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CELERIS™ INSTRUCTIONS FOR USE

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Celeris User Manual

Introduction

The Celeris system uses hardware and software developed entirely by Diagnosys LLC to provide a compact, transportable system for recording any conceivable ERG or VEP test. Diagnosys' industry-leading, patent-pending combined stimulator-electrodes are capable of performing any electrophysiology test, even those previously possible with only custom-built lab equipment such as Scotopic Threshold responses, intensity series, Photopic Negative ERGs, or pattern stimuli.

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Diagnosys UK, LTD, is the single authorized representative in the EU for all Diagnosys devices.

Equipment Covered

D430 Celeris High Throughput Testing System with Computer
D431 Celeris Full-field stimulators
D432 Celeris PERG Stimulator
D239 Platinum Needle Electrodes
D166 Stainless Steel Needle Electrodes

Covers software Version 6+

Disclaimer

Information in this manual is subject to change without notice and does not represent a commitment on the part of Diagnosys LLC nor can it be held responsible for any omissions or errors herein. Diagnosys LLC specifically disclaims any warranties expressed or implied about the fitness of this system for any practical purpose and in no event shall be liable for any loss of profit or other commercial damage including, but not limited to, special, incidental, consequential, or other damages.

Licensing and Copyright

Celeris User Manual

Intended Use

The Celeris Ophthalmic Electrophysiology System is a visual electrophysiology system that is intended to be used by qualified graduate and post-graduate level research personnel or trained technicians to record all types of ophthalmic electrophysiology responses from experimental animals. The Celeris functions by flashing lights or patterns of light at the eye and recording the electrical response from the retina or visual cortex.

Technical Support, Servicing, and Repair

For technical support, contact your local dealer or Diagnosys.

Alterations and repairs may only be carried out by Diagnosys. There are no user serviceable parts.

A return authorization number (RMA) is issued if the Celeris system must be returned for repair. Pack the Celeris system in its original container and return it prepaid and insured. If the original container is no longer available, Diagnosys may be able to provide a replacement.

Maintenance

Electrical shock hazard. No user maintenance is possible.

Inspect all system equipment each time you use it for any external sign of wear or damage (e.g. frayed cables).

Warnings

The Celeris system must be connected exactly as instructed, otherwise the correct functioning or safety of the system may be impaired.

When using electrical appliances, basic precautions should always be followed to reduce the risk of fire, electric shock, and injury to persons, including the following:

DO NOT attempt to repair or replace the AC mains cable that came with your system. Contact Diagnosys for a suitable replacement if needed.

DO NOT attempt to repair or adjust any electrical or mechanical functions on this unit. Doing so will void your warranty. The inside of the unit contains no user serviceable parts. All servicing should be performed by qualified personnel only. Do not disassemble while connected to power, may cause shock or death.

DO NOT operate any appliance with a damaged cord or plug, after the Celeris malfunctions, or if it has been dropped or damaged in any manner. Return the Celeris to Diagnosys for examination, electrical or mechanical adjustment, or repair.

Celeris User Manual

Technical Specifications and Ratings

Console Buttons and Status Lights

A: Amplifier inputs
B: Run
C: Pause/Preview
D: Stop/Check Impedance
E: Toggle Safety Light/Set Safety Light Intensity
F: Warming Table Power Indicator
G: Warming Table Below Temperature Indicator Light
H: Warming Table Above Temperature Indicator Light (error state)

Figure 1 - Console control buttons and status lights

Dimensions and Weight

Celeris (height to tabletop): 15.7 x 6.9 x 5.3 inches (399 x 175 x 135 mm)
Celeris heating table: 6.1 x 4.0 inches (155 x 102 mm)
Celeris weight: 10 lbs (4.5 kg)

Power

Power Supply Voltage Input: 100 ~ 240 VAC 50/60Hz, 1.0A
Power Supply Voltage Output: 12V
Power consumption in use: 24 W

Power Supply and Cord

The plug end of the power supply is to be plugged into a grounded outlet. The grounded proprietary 12V power input is plugged into the three-pin jack, matching the red dot on the Fischer connector to the red dot on the console.

Figure 2 - Celeris Power Supply & connection to console

In the event of damage or a misplaced power cord, do not attempt to replace the power cord; an inappropriately labeled cord might not provide the proper insulation, be rated for the correct current, or be short enough to minimize electric interference and the risk of trip hazards.

For continued protection against fire of shock, replace the provided detachable power supply cord only with a line cord supplied or approved by Diagnosys.

Input/Output Jacks, Power Button

Custom HDMI-connector pattern generator ports x2 (①,②)
Proprietary Ultimate 7-pin Full-field Stimulator jacks x2 (③,⑤)
USB-A power-only output jacks x2 (④)

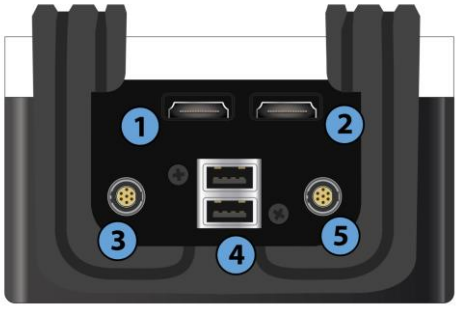


Figure 3 - Celeris connections, left front panel

Proprietary 12V power input (A)
Main power button (B)
High-speed (USB2.0) USB-B I/O (C)
DIN4 jack for gooseneck red LED light (D)

Output rating:
USB 1: 5V, 500 mA MAX
USB 2: 5V, 500 mA MAX
DIN4: 12V, .61A MAX

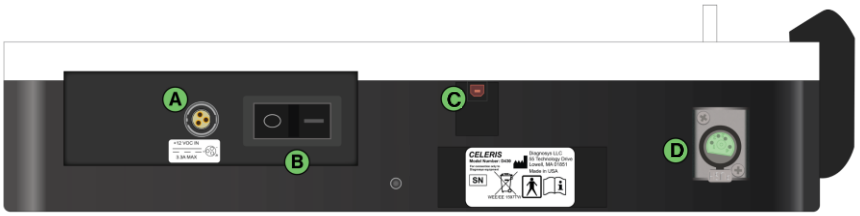


Figure 4 - Celeris connections, back panel

Safety Standards

The Celeris System meets the following standards:

- IEC 61010-2-101
- IEC 61010-2-010
- IEC 61010-1
- IEC 61326-1
- FCC 47CFR 15B clB
- EN 61010-1:2010

Conforms to:
- UL STD 61010-1
- UL STD 61010-2-010

Certified to:
- CSA STD C22.2 # 61010-2-010
- CSA STD C22.2 # 61010-1-12

Intertek
5002629

System Specifications

Note: See product brochure for updated system specifications

Testing speed	Test 10 to 20, or more, rodents per hour (depending on your protocol length)
Animal setup time	Seconds
Test species	Mice, rats, gerbils, guinea pigs and rabbits
Amplifier	• 2-channel, fully differential, 32-bit amplifiers have 1 nV resolution over a 4V input range, which provides the highest resolution in the industry • Ultra-low noise • 5V input range, ensures amplifier will not saturate • Common mode rejection ratio (CMMR): >100 dB
Rodent platform heater	Temperature: 99°F / 37° C, ± 1°C
Overhead safety light	Red (660 nm) and infrared (940 nm) LEDs
Data reporting	Exports natively to .csv, .txt, or to clipboard for easy import into data processing software such as Excel, SIGMAPLOT, or MATLAB
Platform cleaning	Flat tabletop covered with waterproof membrane makes cleanup simple and easy
Camera option	Infrared camera available
Protocols supported	Photopic and Scotopic ERG testing C-Wave ERG Photopic Negative ERG Scotopic Threshold Testing Flash VEP Pattern ERG and VEP

Stimulator options	Bright: .0003 cd.s/m^2 through 700 cd.s/m^2 (extended range available) .009 - 6000 Dim: 10^{-7} cd.s/m^2 through 10^{-1} cd.s/m^2, $5x10^{-7}$
Computer operating system	Windows 10 Professional

Operating Environment

This system is designed for:

- Indoor continuous use.
- Altitude up to 2000 meters
- Temperature range of 5-40° C.
- Maximum relative humidity 80% for temperatures up to 31° C, decreasing linearly to 50% relative humidity at 40° C.
- No hoar-frost, dew, percolating water, rain, solar radiation shall be present, as the operating environment should be indoors only.
- Mains supply voltage up to +/- 10% of the nominal voltage
- Transient overvoltages up to the levels of overvoltage category 2.
- Temporary overvoltages occurring on the mains supply.
- Intended environment not to exceed pollution degree 2.

The Celeris will operate safely and effectively under environmental conditions too extreme for experimental animals to survive. For best recording conditions, Diagnosys recommends optimizing environmental conditions to minimize mouse stress. Temperatures of ~18-23°C (65-75°F) with 40-60% humidity are recommended by The Jackson Laboratories.[1] Long-wavelength (ideally LED-based >680 nm) red lighting can be used to minimize light adaptation during scotopic testing but should be minimized. Noise and vibrations are to be minimized as they can cause stress to the animals.

Although no special electrical shielding is required, the Celeris should not be placed in an electrically noisy environment; large machinery such as cage washers, heavy-duty refrigeration equipment, or elevator motors may introduce electrical interference into the signal. Power strips/surge protectors may induce noise. The Celeris and associated computer should have a dedicated power outlet if possible. Keep electrical cables as far from the Celeris as possible to prevent introducing electrical noise into the signal.

You will need all other facilities normally required for visual electrophysiology testing, such as access to running water and the ability to control room lighting.

System Setup

Assembly Recommendations

- Because the AC mains cable is the disconnecting device, make sure that the Celeris is positioned so that it can be easily unplugged from the wall if necessary.
- The Celeris must be plugged into a grounded outlet for proper protective earthing.
- The Celeris has no special ventilation requirements. If you are using gas anesthetics, ensure that the Celeris is in a well-ventilated area commensurate with the special requirements of the anesthetic delivery system in use.
- Safety in incorporating the system is responsibility of the assembler.

[1] The Jackson Laboratory. (2015). *Mouse Room Conditions*. [online] Available at: https://www.jax.org/jax-mice-and-services/customer-support/technical-support/breeding-and-husbandry-support/mouse-room-conditions.

- For continued safe operation, use only accessories manufactured or approved by Diagnosys LLC.

Instructions for Use

Setting up for a test

Animal Preparation

This should be done before running a test.

Note: this protocol should be performed in accordance with your institution's Animal Care and Use Committee regulations and may be subject to institutional approval.

If any part of the testing will be done in Scotopic (dark-adapted) conditions, the animal should be left in a light-proof dark room overnight. Be sure to review your institution's policy regarding overnight housing in advance.

1. Anesthetize the animal. The most common anesthetic for rodent electrophysiology is an intraperitoneal (IP) injection of ketamine and xylazine, as some gas anesthetics have been found to affect the ERG response. When using the ketamine-xylazine cocktail, it is important to weigh the animal first to calculate the correct dose. Improper dosing can lead to the death of the animal, or the animal failing to become fully anesthetized. Under optimal anesthesia, the breath rate should be ~55-65 breaths per minute, and there should be no muscle movement or corneal reflex. Under insufficient anesthesia the breath rates will be greater than 70 breaths per minute and the corneal reflex will be intact. Too deep anesthesia will result in <50 breaths per minute and heavy breathing (gasping).[2] See below for suggested anesthetic dosages.
2. Maximally dilate the animal's pupil. Typically, a combination of Phenylephrine HCl and either Tropicamide or Atropine is used. Allow the drops to sit on the eyes for approximately 2 minutes before wicking excess fluid from the eye in order to prevent inadvertent aspiration. Aspiration can lead to the death of the animal. For testing that requires long recordings, such as c-wave or DC-ERG responses, corneal anesthetic such as proparacaine is recommended. Hydrating drops must be applied directly to the eye to prevent corneal drying and subsequent cataracts. A **.3% Hypromellose lubricating gel solution is best.** Saline or artificial tears are too thin to stay on the eye and will need frequent reapplication to keep the eye hydrated. 2.5% (gonioscopic) hypromellose is too thick to sufficiently hydrate the eye and should not be used.
3. Optional – you may trim the whiskers if desired. This is not required or necessary but may make it easier to place corneal electrodes, particularly the pattern stimulator electrode.

Suggested Anesthetic Doses (Mice)

- Ketamine @ 80 mg/kg [75 – 150] + Xylazine @ 20 mg/kg [16-20]. Some animals, including particularly young wild types, may require additional ketamine. Suggested range is indicated in square brackets following the suggested dosage.

[2] Ewald, A. J., Werb, Z., & Egeblad, M. (2011). Monitoring of Vital Signs for Long-Term Survival of Mice under Anesthesia. *Cold Spring Harbor Protocols*, *2011*(2), pdb.prot5563-pdb.prot5563. https://doi.org/10.1101/pdb.prot5563

- Ketamine at 50mg/kg [50-75] + Medetomidine at 1.0 mg/kg [0.5 to 1]. Some animals, including particularly young wild types, may require additional ketamine. Suggested range is indicated in square brackets following the suggested dosage.

Environmental Preparation

Cover or turn off any light sources in the room. Light sources covered with red filters may be used but may affect dark adaptation, due to the wide passband of many filters. The room should be completely dark. Ensure that the Celeris is turned on and that the heating pad has been permitted to come up to temperature. A recovery area for animals recovering from anesthesia should be prepared in advance.

Software Preparation

From the launch page of the software, select the correct Examiner from the dropdown (for further information on adding or modifying examiner names to the software, see Local Configuration Settings) and press the **Start** button.

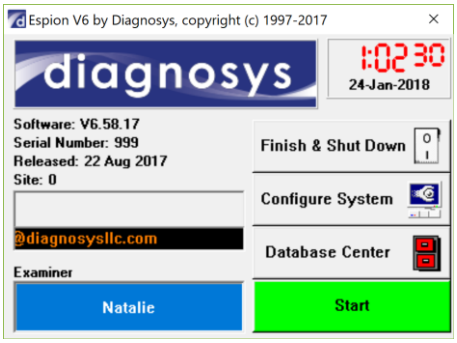


Figure 5 - Launch Page

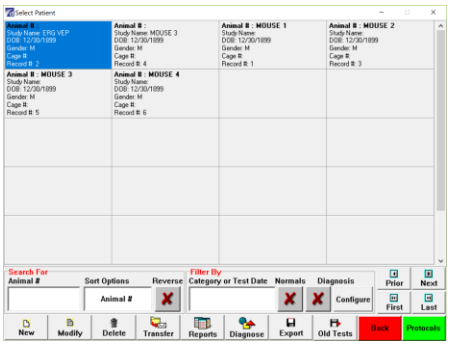


Figure 6 - Subject Selection Page

This will lead you to the subject selection page. By default, each 'patient' represents one animal. Default field names for each animal subject are Animal Number, Study Name, DOB, Gender, and Cage Number. For further information on customizing field names, see Local Configuration Settings.

Create a new subject by pressing 'New' and inputting identifying fields, or searching for and selecting an existing subject. When the correct subject is selected, press **Protocols** to advance to the protocol selection page.

Select the test you wish to run from the list. For additional information on customizing test protocols, see Creating and Modifying Protocols. When you have selected the test protocol, press **Run** to enter the runtime menu.

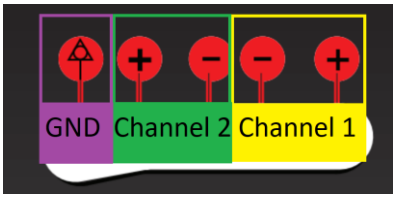


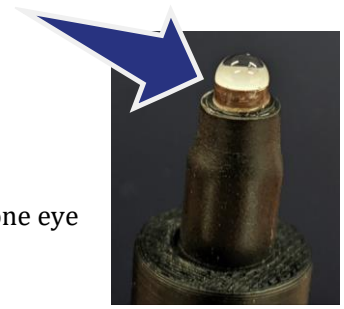
Figure 7 - Channel labeling guide

Diagnosys-supplied protocols will display text prior to entering the runtime menu explaining how to set up the animal.

Placing the Electrodes

Protocols labelled TOUCH/TOUCH ™

TOUCH/TOUCH protocols are protocols that take advantage of the patent-pending stimulator/electrode combo unique to the Celeris. *TOUCH/TOUCH* protocols stimulate one eye at a time and use the fellow, unstimulated eye as the reference.



To set up an animal for a *TOUCH/TOUCH* protocol, deposit some of the .3 % hypromellose solution in the cup of each electrode. Ensure that the cup is full of wetting solution to the point of overflow. (See image, right). There should be sufficient solution in the electrode to keep the cornea hydrated during testing.

Figure 8 - electrode with gel in cup

Place the electrodes on the eye. The electrodes should be placed symmetrically and aim down the optical axis of the animal. See Figures 10-14 below as examples.

If you need additional height for your electrode, magnetic T-bars can be used in conjunction with the magnetic posts.

Figure 9 - T-bar increasing the height of the electrode

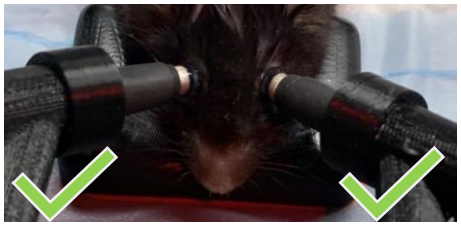


Figure 10 - Both stimulators positioned correctly

Figure 11 - Left stimulator correct, right stimulator too far back

Figure 12 - Left stimulator too far forward, right stimulator correct

Once the electrode is placed on the eye, ensure that the entire eye has been covered in wetting solution, and that the wetting solution contacts the silver band of the electrode. You can add more wetting solution at this stage if necessary.

If you will be using gas anesthetic, make sure that the animal is positioned securely in the nose cone prior to placing the electrodes on the cornea. Figures 13 and 14 below show mice with a *TOUCH/TOUCH* electrode setup. Note that the nose cone for gas anesthetics is an optional component. Note also that a third ground electrode is optional in this setup. See Figure 7 on preceding page for Channel labeling guide.

Figure 13 - The TOUCH/TOUCH Setup

Figure 14 - The TOUCH/TOUCH Setup with gas nose cone

When plugging a *TOUCH/TOUCH* protocol into the Celeris, imagine bisecting both the rodent and the Celeris along the midline of the animal, as seen in Figure 15 below. Every plug/input on the right side of the animal should stay on the right; every plug/input on the left side of the animal should stay on the left. See Figure 15 below for how the electrodes should be plugged into the amplifier for a *TOUCH/TOUCH* protocol. The right eye should be plugged into Channel 1+; the left eye should be plugged into Channel 1-. If a ground is used, it should be plugged into the GND input ( ).

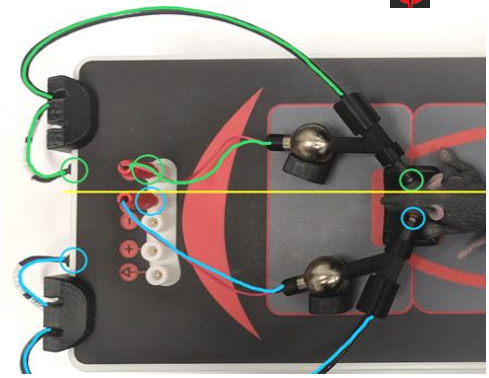


Figure 15 - Amplifier Setup of a TOUCH/TOUCH™ Protocol

The electrode/stimulator contacting the animal's **right** eye (green) is plugged into the amplifier input 1+ (on the animal's right) and the stimulator port to the animal's right. The electrode/stimulator contacting the animal's **left** eye (blue), is plugged into 1- (the channel 1 input closest to the animal's left), and the stimulator port to the animal's left.

Protocols labelled ACTIVE/REF/GND

ACTIVE/REF/GND protocols are traditional protocols that use binocular stimulation, a shared reference electrode, and a ground electrode to reduce or eliminate mains interference. Typically reference electrodes are placed either subcutaneously in the forehead area, or a bite bar placed in the mouth. Ground electrodes are typically needle electrodes placed subcutaneously in the tail or the hindquarters near the tail. The right eye will be plugged into Channel 1+, the left eye will be plugged into Channel 2+, the shared reference will be plugged into both Channel 1- and Channel 2-, and the Ground into the ground input.

Simultaneous ERG and VEP Protocol

The simultaneous ERG and VEP protocol supplied by Diagnosys uses input channel 1 for the corneal/ERG recording and input channel 2 as the VEP/cortical recording.

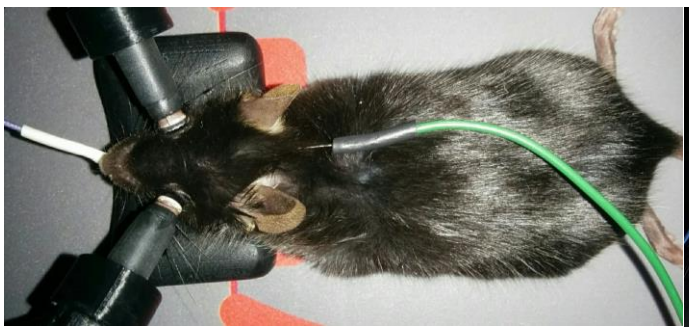


Figure 16 - Setup for combined ERG/VEP. GND in tail (not shown)

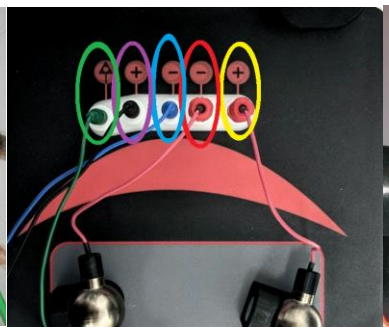


Figure 17 - Layout for ERG/VEP protocol

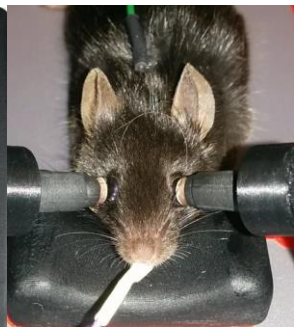


Figure 18 – alt view of setup

The animal's right eye stimulator/electrode is plugged into Channel 1+ (indicated in yellow in Figure 17 above). The animal's left eye stimulator/electrode plugs into Channel 1- (red). A VEP reference needle electrode should be placed in the snout or cheek (as seen Figure 14 above). The plug for the VEP reference should go into channel 2- (indicated in blue in Figure 17 above). The active VEP electrode should be at the midline at the back of the head and is plugged into 2+ (indicated in purple). The final ground electrode is placed in the tail or hindquarters near the tail and is plugged into the input circled in green in Figure 17. (See Figures 16 - 18).

Setup Tips and Tricks

Animal Placement

Ensure that the animal is stretched to its full length and not "bunched up". If the animal is hunched over the headrest, when it breathes its head will move against the electrodes and cause noise and baseline disruption. Don't injure the mouse by stretching him! It is enough to ensure that when he breathes, his head does not move.

One technique for placing the mouse is to put him down a little before the headrest and **gently** drag him forwards onto the headrest.

Another technique is to place the mouse a little forward of the headrest and use the tail to pull him backwards into position.

The key in both techniques is **gentle**. Do not induce spinal damage – do not stabilize his head when pulling backwards on the tail. Simple allow the mouse to extend to its full length.

Once the electrode is perfectly placed, ensure that the tension from the stimulator cable has not pulled the electrode off the eye or out of position when you let go of the electrode.

See the following images as an example.

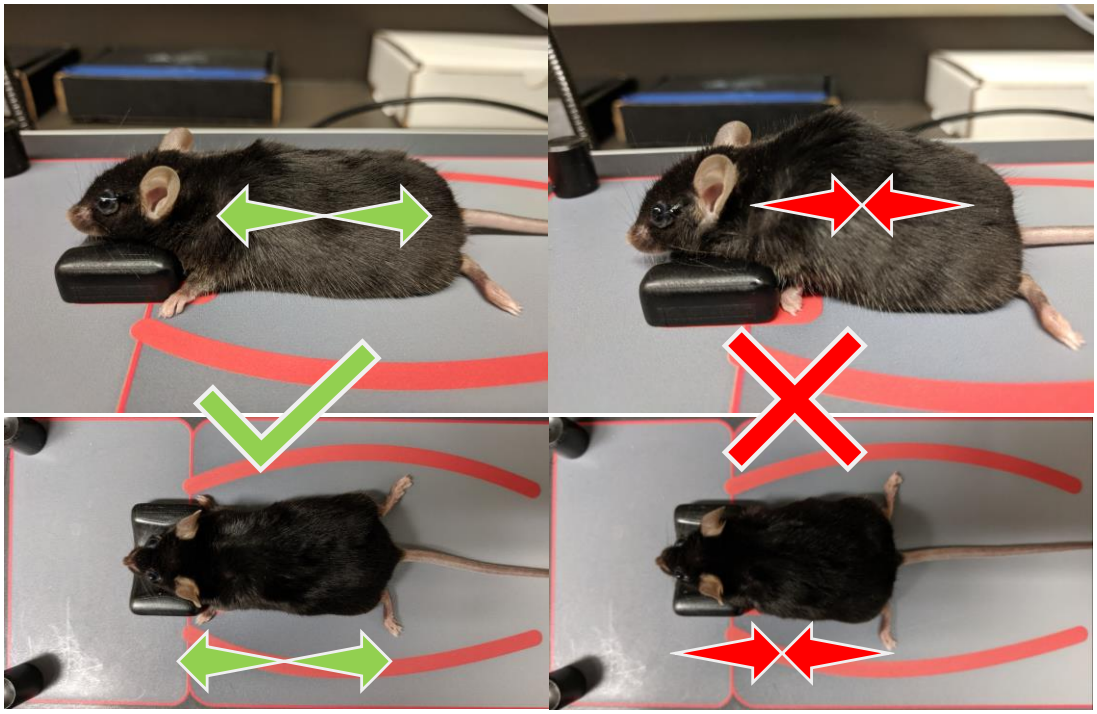


Figure 19 - Animal positioning

Ground electrode

- The ground electrode is a non-recording electrode that is used to prevent electromagnetic noise (for example, from power cables) from interfering with the signal. Common placement sites include the tail, or the hindquarters near the tail. While there is no electrophysiological advantage to either site, Diagnosys recommends placing the electrode on the hindquarters near the tail, as the skin of the tail is typically tougher and will blunt an electrode more quickly.

- The Celeris has been hardened against electromagnetic interference, and you may find that adding a ground electrode is not necessary to protecting against mains interference, particularly in TOUCH/TOUCH protocols. TOUCH/TOUCH protocols by default assume no ground electrode is placed.
- In addition to preventing electromagnetic noise from interfering with the signal, the ground electrode permits the impedance of each active electrode to be assessed independently. Without a ground, only the average of the impedances of the two electrodes can be determined and therefore both eyes will always show the same value.

Full-Field Stimulator electrode

- Make sure that the eye has been wetted before placing the electrode.
- After the electrodes have been placed, ensure that all visible parts of the cornea have been thoroughly covered with wetting solution. Add more solution directly to the eye if necessary, to ensure corneal hydration.
- Ensure that the electrodes contact the cornea directly – making contact solely through the wetting solution will decrease amplitudes as the solution dries.
- Do not put pressure on the eye! Putting pressure on the eye will increase intraocular pressure and consequently reduce blood flow to the retina, resulting in decreased amplitudes. Ensure that the electrode is touching the cornea very gently.
- See Placing the Electrodes section for images and examples.

VEP Electrodes

- Needle electrodes can be used as active VEP electrodes. Subdermal needle electrodes are relatively non-invasive and more convenient to operate. However, VEP waveforms are affected by electrode positioning. For increased reproducibility and larger amplitudes, surgically implanted screw electrodes placed above the visual cortex can be used. This section addresses the placement of needle electrodes.
- The active needle electrode needs to be placed subcutaneously above the area of the visual cortex. Start by pinching and lifting the skin between the ears of the mouse and sliding the needle under the skin towards the nose and horizontal to the skull. Ensure that the needle is fully in under the skin so that the tip of the needle is past the ears.

Figure 20 - VEP Needle Electrode Placement

Pattern Stimulator Electrode (Optional component)

- **Ensure that the electrode is clean before use!** See the cleaning section for details.
- **Important: when the stimulator is placed correctly, you should be able to see a bit of the cornea above the stimulator**. If you are unable to visualize the cornea, you may be putting too much pressure on the eye and may need to pull the stimulator back slightly.
- Total dark adaptation is required. Dark adapt the mice for a **minimum** of 3 hours (overnight dark adaptation is typical). Rats should be dark-adapted for a minimum of 1 hour.
- Despite relying on visual acuity, this is a **dilated** test! The stimulator imposes its own aperture on the eye, so make sure that the animal is maximally dilated, and that the electrode is positioned so that it's pointing down the optical axis. See positioning images below for guidance.
- Make sure that the eye is clear and without cataracts before starting the test! This test is dependent on visual acuity and vision-compromising cataracts will adversely affect the response.
- Make sure that there are no whiskers stuck to the eye. This can interfere with the optics and affect the response.
- If you are not getting a response – look in the stimulus viewing window directly above the stimulator aperture to determine that the stimulus is running.
- Be careful with the amount of hypromellose/wetting solution used. If placed directly on the cornea rather than the electrode, too much can make it difficult to visualize the eye in the dark.
- **In the protocol parameters, ensure that the protocol distance is set to 340 mm – this is the correct distance for the PERG stimulator**. Prior revisions of Diagnosys protocols had this set to 1100 and should be updated. If necessary, contact Diagnosys to ensure you are running the most current PERG protocol.

Binocular Pattern Stimulator Protocols (Optional component)

- If using two pattern stimulators simultaneously in order to record binocular pattern ERGs or pattern VEPs, Diagnosys has binocular pattern protocols available. These protocols rotate the stimulus 90 degrees. This allows for the stimulators to be rotated 90 degrees. Raised aluminum blocks are available to raise the height of smaller animals.

Figure 21 - A Mouse on a raised platform performing a binocular pattern ERG

Monocular PERG Positioning Images

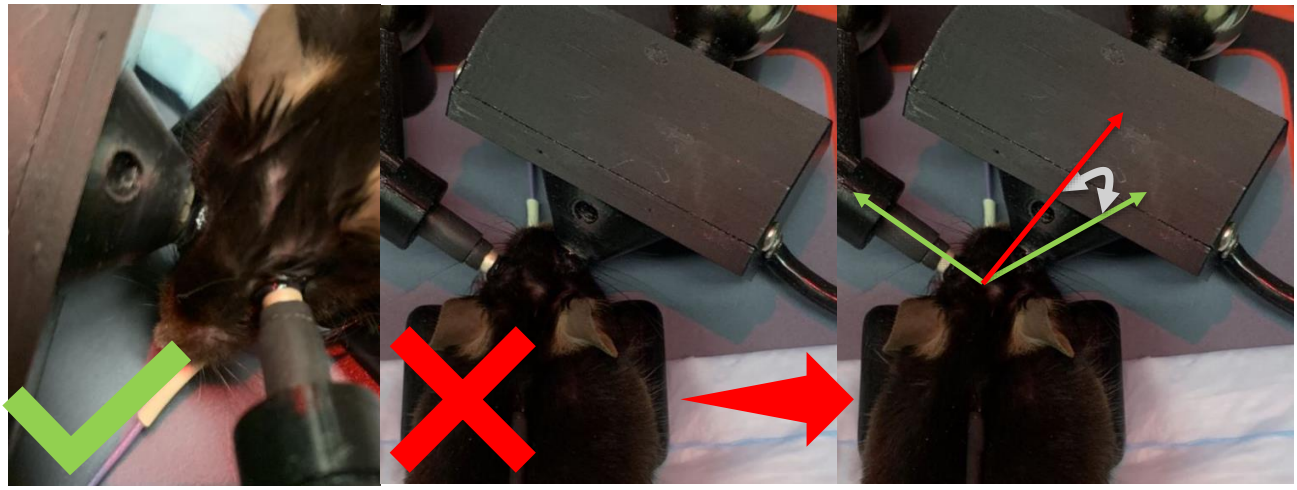


Figure 22 - PERG Positioning

In good positioning (✓), the cornea is visible over the tip of the PERG stimulator, and the stimulator is pointed down the optical axis.

In the poor positioning (✗), the stimulator is positioned too far forward, and the cornea is not visible. There is too much pressure being placed on the eye, and the mouse cannot see the stimulus. In the example above, the center of the PERG stimulator should align with the optical axis of the mouse (green arrow) but is too far forward (red arrow).

Runtime Menu Controls / Celeris Control Buttons

This is the runtime menu of the Diagnosys software:

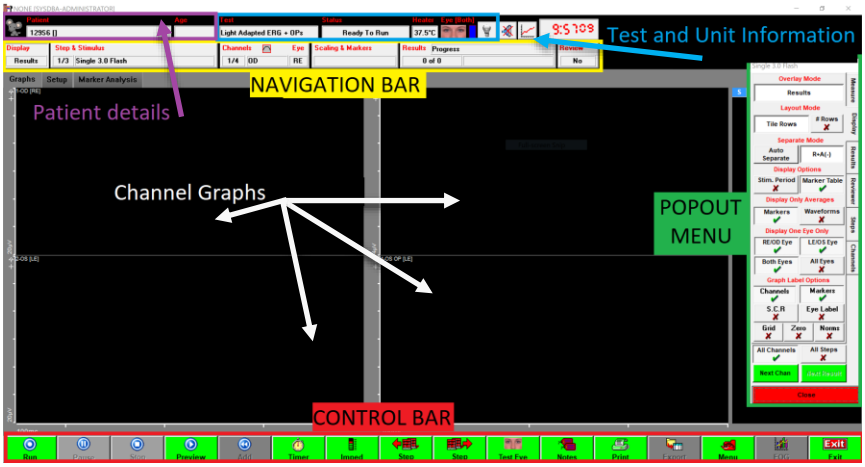


Figure 23 - Runtime Menu

The **Patient Details Bar** includes the identifying information filled out in the subject record, such as study name, animal number, cage number, and age (in years).

The **Test and Unit Information** includes the name of the test being performed; the test status (ready to run, paused, previewing, waiting for an adaptation timer to complete, or waiting for an interstimulus delay to end. It also reports the current measured heater temperature (target temperature is 37 °C, ±1) and which eye is being tested by this step of the protocol. A small lightbulb icon at the top controls the safety light on the Celeris.

The **Navigation Bar** at the top controls the popout menu at the right. Pressing any of those buttons will cause the corresponding popout menu to appear; depressing the buttons will cause it to close. The popout menu can also be navigated using the tabs on the right edge.

Figure 24 - Navigation Bar

There are six buttons on the navigation bar. The first button is **Display**, which controls the display settings for the test. The **Step & Stimulus** menu reports current stimulus and acquisition parameters; these are primarily used during new protocol creation, not typical use. The **Channels & Eye** menu houses the 50/60 Hz line filter; it also reports the current filter and auto-rejection window settings. The **Scaling and Markers** button allows you to adjust marker placement or change channel scaling as necessary. The **Results** menu allows you to delete or toggle the visibility of recorded results, reject individual artifacts, change the color of responses, and create grand averages. **Review** is primarily used in multi-site clinical trials and allows a reviewer to analyze each step of each test for changes or problems in order to alert safety committees; it is not commonly used in a research or clinical setting.

The **Control Bar** at the bottom of the page controls the test. Most of these buttons have their functions duplicated by the control buttons on the Celeris. By default, buttons that can be pressed are green; buttons that are active are red; buttons that cannot be used at the moment are greyed out. Figure 25 below shows a Control Bar in the ready state; since the software is not doing anything (and there are no results recorded), the test cannot be paused or stopped, and there is no data to export or add to. The EOG button is also greyed out; the EOG is a test of the RPE layer of the human eye and is not relevant for animal applications. Figure 26 below shows a control bar in the Preview State – note that the array of available commands has changed now that the amplifiers are actively sampling data. The color of the control bar buttons can be customized in the Local Configuration Settings.

Figure 25 - Control Bar; Ready state

Figure 26 - Control Bar; Preview state

The **Run** button will start the test. Most Diagnosys-provided protocols are *automatically sequenced*, which means that when the test is started, it will automatically advance through the protocol until completion. Manually sequenced protocols will require the operator to use the Step button to advance through the protocol. The **Pause** button will pause a running test, and **Stop** will stop the recording. **Preview** will begin sampling baseline data. The **Add** button will add a full complement of sweeps to the selected result.

The **Timer** button will allow you to see or control the timer in a protocol that has adaptation timers defined.

Imped will allow you to check the impedance (resistance; essentially a measure of electrode connection) of the setup. Ideal impedances are between 5 and 15KΩ. Most Diagnosys-supplied protocols will automatically launch the impedance checker when you start the test.

The **Step Forward** and **Step Back** buttons allow the operator in a manually-sequenced protocol to advance through a test. The step you are on (and how many steps there are in the test) are updated in the Step and Stimulus button in the navigation bar.

Test Eye is not used in animal applications.

Notes allows you to write free-form notes (for example, "animal woke up partway through exam") that will appear on printouts of the test.

The **Print** and **Export** buttons allow you to print results, either to hard copy or a PDF printer, and to Export graphic or ASCII data to third party software.

The **Menu** button launches the popout menu.

The Celeris is not capable of performing the human-only EOG test, so the **EOG** button will remain greyed out.

The **Exit** button allows the operator to exit the test. When exiting the test, the operator will be prompted to save. A 'no' response will require confirmation. If a test is discarded in error, contact Diagnosys for help in retrieving the test data.

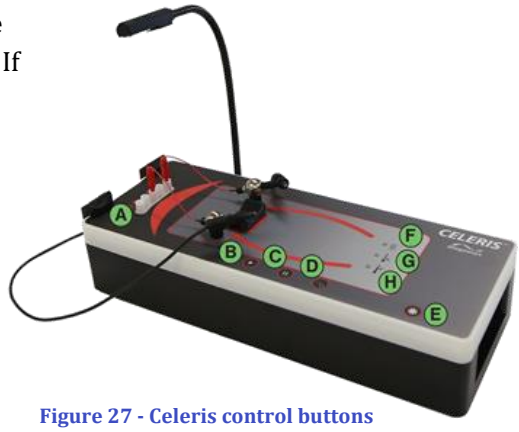


Figure 27 - Celeris control buttons

The system can also be controlled by the buttons on the Celeris itself.

A: Amplifier inputs
B: Run button – starts the test.
C: Pauses a running test/Starts preview when in ready state
D: Stops a running test or previews/Check Impedances in ready state
E: Toggles safety light/ press and hold to adjust safety light intensity
F: Warming pad power indicator
G: Warming pad below target temperature indicator (still warming)
H: Warming pad above target temperature indicator (error state)

Running a Test

Press **Run** to begin the test. Automatically sequenced protocols (such as the ones Diagnosys provides), will automatically advance through the test protocol until the test is complete. Manually sequenced protocols will require the operator to use the **Step Forward** button to advance through the test, and to press **Run** at the beginning of each step.

In-test Troubleshooting

Problem: Mains interference

Solutions: Try to ensure all power cables are physically kept away from the Celeris. Ensure that the outlet the Celeris is plugged into is grounded. If using a laptop, tablet, or other battery-powered computer to drive the Celeris, try unplugging the computer from the wall to see if it is producing the noise. To enable the mains/line filter, press the **Channels/Eye** button at the top of the runtime menu and toggle the **50/60 Hz Line Filter** from the popout menu that appears.

Figure 28 - Channels Eye popout menu

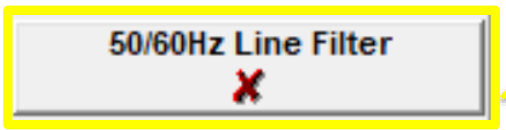


Figure 29 – Line filter button

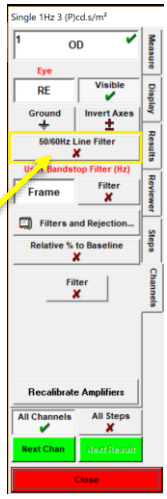


Figure 30 – Channels Eye popout menu

Problem: Respirations and/or heartbeat appearing in the trace

Occasionally, heartbeats and respirations may contaminate a response. While not typically an issue, respirations can cause very large deviations in signal (see Figure 31, right, which is scaled at 800 uV/division and recorded for 10 seconds). When using gas anesthetics, large gasping respirations can indicate too deep a level of anesthesia and adjusting the gas mixture may help.

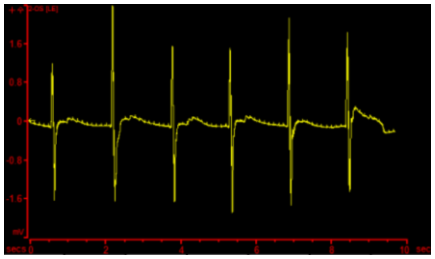


Figure 31 - Respirations (large deviations) and heartbeats (small spikes on trace)

One way to reduce this is to adjust the positioning of the mouse so that the animal is "stretched out" and not "bunched up." Deviations occur as the animal moves its head and the electrodes move against the eye. Ensure that the animal is not bunched over the headrest so that breaths expand the abdomen without the head. See Animal Positioning section for more information.

Adjusting electrode placement (particularly reference electrodes in ACTIVE/REF/GND protocols), and careful use of wetting solution can also help minimize these biological artifacts. The use of thinner wetting solutions (such as saline) can require frequent re-wetting of the eye, which can cause the fur of the face and chest to get wet, creating a conductive path between the heart and the ERG electrode. Using smaller amounts of thicker solutions can prevent this from occurring. If you are seeing respirations or heartbeats in your trace, try adding more gel and re-placing the electrodes.

Problem: No signal when one is expected

If you are not getting an ERG response when you would expect one, ensure that the stimulus is being presented and that the flash intensity (if a full-field stimulus) is not outside the specifications of your stimulator, or that the pattern stimulus is visible through the stimulus viewing window immediately above the electrode/stimulus aperture. If the stimulus is being presented, ensure that the electrodes are correctly positioned and plugged into the amplifier, and that the animal's eye is clear and free of cataracts; this can also occur if too much hydrating gel is used, or if the wetting solution used is a liquid and not a gel. If the wetting solution from the right eye makes contact with the wetting solution from the left eye, the electrical bridge between the two eyes will make the response from both eyes the same, and zero any response. This is of especial concern in very small (young) mice.

If you still are not getting a response, try another animal – sometimes mice that should be normal aren't!

Problem: High unexpected inter-ocular variability

The main cause of variability in ERG testing is incorrect electrode placement. Make sure that the electrode is placed properly (refer to Setup Tips and Tricks for further information on electrode placement). If the variability persists after re-placing the electrodes, try "hot swapping" the stimulus electrodes – unplug them from all connections and switch which eye they are stimulating. **If the eye with the higher response stays the same** the problem is likely with the animal – try testing the next animal to see if the problem persists. **If the larger response switches to the other eye**, contact Diagnosys for further troubleshooting.

Problem: Test is running and running but doesn't appear to record anything

If the test is running and does not appear to be progressing, the auto-reject window may be enabled and set too tightly. In the Results button of the navigation bar, the results tab will report the number of traces recorded (0 of 6 in Figure 32, right) out of the number of responses desired (6), and the number of responses rejected as being outside of the auto-reject window (5). The auto-reject window can be adjusted by pressing **Channels Eye**, and adjusting the auto-reject window or disabling the auto-reject entirely. Press **Ok** to save, and be sure to make changes to "all real" channels.

Figure 32 - Auto-rejecting traces

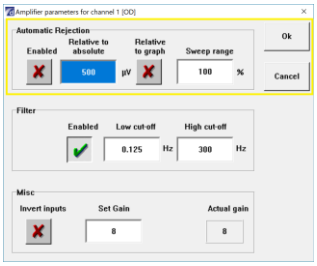


Figure 33 - Automatic rejection parameters

Printing, Saving, and Exporting Data

Your data is being saved to the hard drive during testing. To save data to the database, press the **Exit** button at the bottom right at the end of the test.

Figure 34 – Exit (and save)

Figure 35 - Print

When exiting the test, you will be asked if you wish to save the test to the database. A **No** answer will require confirmation. If you discard the test in error, contact Diagnosys support for instructions to retrieve the test.

To print the test, press the **Print** button on the Control bar. The print options tab on the side of the preview window will allow you to adjust the print settings and save templates to recall later.

To export data, press the **Export** button. You can export ASCII data to either a .csv or .txt file or copy the data directly to the clipboard. Graphics (such as graphs, or images of marker tables) can also be copied directly to the clipboard.

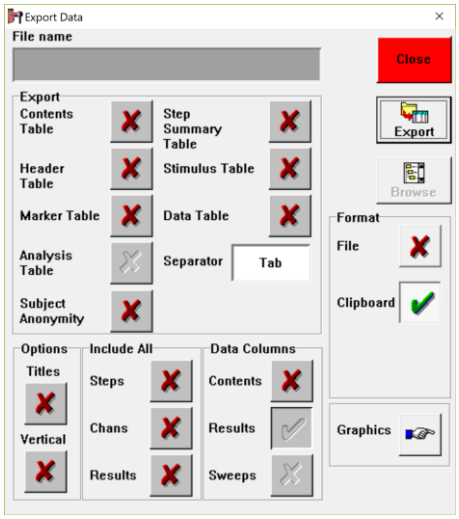


Figure 36 - Export data

Contents Table: Tells you the size (in cells) of the data exported.

Header Table: Includes protocol data such as protocol name, number of channels, stimulator type, time and date of test, and subject details.

Marker Table: Provides the recorded marker values for the loaded test.

Analysis Table: N/A to animal testing, exports the Arden ratio for EOG testing.

Step Summary Table: Provides information on data recorded in each step, including number of responses recorded, number of responses rejected, and time and date stamps on recordings.

Stimulus Table: Provides information about stimulus parameters for the test.

Data Table: Provides X and Y axis values for each data point on the waveform.

Separator: When exporting to a file, data delimiter can be Tab, comma, colon, semicolon, or space.

Titles: Useful when exporting multiple tables at once; will title each section of the spreadsheet.

Vertical: Transposes columns and cells; was added as a workaround for Excel 2003 and earlier which would not permit more than 256 columns.

Include All: If selected, will include all Steps/Channels/Results. If unselected, will only export the current step/channel/result.

Data Columns: Requires the Data Table be enabled. Contents includes information such as which Channels, Results, and Trials columns are included. Results includes averaged responses. Sweeps includes the individual responses that make up each average.

File/Clipboard Format: Allows the user to determine whether the data is copied to clipboard or exported to a file for later import into 3rd party data processing software. Note: when exporting to a file, the user must use the Browse button to manually set a filename and directory.

Exporting Graphics: Clicking each button will place the associated graph on the clipboard for pasting into 3rd party imaging or presentation software.

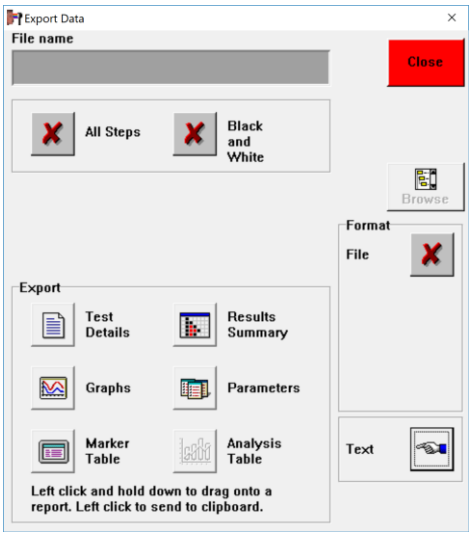


Figure 37 - Export Graphics

Loading Saved Tests

Previously saved tests can be accessed from the subject selection page by pressing the **Old Tests** button. This will display a list of all tests recorded under that subject record. Saved tests can also be accessed by selecting a subject, pressing **Protocols**, and pressing the **Old Tests** button. This will display a list of tests associated with that subject *and* that protocol. Filters for Subject and Protocol can be unchecked at this window, allowing a list of all tests recorded associated with any subject/any protocol or any subject/specific test protocol.

Creating and Modifying Protocols

Each Celeris comes with a pre-programmed list of protocols. When creating new protocols, Diagnosys strongly recommends using an existing similar protocol as a template, making a **Copy** of the protocol using the Copy button from the protocol selection page, and **Modify**ing it to suit your needs.

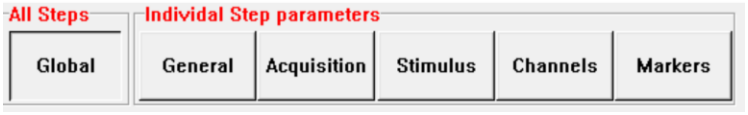


Figure 38 - Protocol parameter categories

Global parameters affect all steps. This includes whether a step is automatically sequenced or requires the operator move from step to step manually; screen display options; default printer and export options. All other parameters affect only the step currently selected at left.

General parameters include information such as the name of the step; how many results (averages) are taken per step; whether there is an adaption timer associated with that step; and if the stimulation alternates eyes between sweeps.

Acquisition parameters include how many data points are taken per second, and how long the system records both before and after the flash.

Stimulus parameters include items such as flash color, flash intensity, background color and background intensity for the full-field stimulators. Waveform stimuli such as sawtooth stimuli, sine waves, or square waves are also available. For pattern stimulators, checkerboards, bars, and gratings are available stimuli, and contrast and timing characteristics can be customized.

Channels allows you to set high and low bandpass filters, which eye is associated with each channel, and virtual channels (digital copies of input channels that can be filtered in different ways).

Markers allow you to set up markers for each individual step, including the automatic placement algorithm and whether the marker reports the absolute value or a value relative to the location of another marker.

Additional protocols, or help in customizing them, can be had by contacting Diagnosys support or found on the Diagnosys webpage.

Calibration, Cleaning, Disinfection, and Storage

Calibration by end users is not possible. To calibrate your Celeris stimulators, contact Diagnosys. Diagnosys recommends calibrating full-field stimulators on an annual basis. Pattern stimulators do not require calibration.

To clean the Celeris console, unplug the power supply and use tape to cover any openings. Spray with isopropyl alcohol or other disinfection solution and wipe dry. Alternatively, disinfectant wipes may be used.

If liquid enters the electrode well, immediately wipe the spillage to prevent damage to the electrodes.

Caution: improper cleaning may lead to damage of the electrodes!

Caution: when lifting electrodes, be sure to lift from the magnetic ball to reduce strain on the electrode.

Cleaning Full-field Stimulator Electrodes

Celeris stimulators should be cleaned after every recording session. **You should not have to clean the electrodes after every animal.** If the electrodes are kept wet during testing, there will be no decrease in performance. To clean the electrodes, place the tip of the stimulator **pointing down** under warm running water to loosen the hypromellose solution. An alcohol wipe, Kim wipe, or moistened cotton-tipped applicator may be used with care to remove the solution from both the cup and the rim of the electrode. **Do not apply too much pressure as this may lift or bend the silver**, rendering the stimulator inoperable and increasing the likelihood of inducing a corneal abrasion. Note in Figure 41 below the silver of the electrode has been pulled away from the light pipe. This can happen if you pinch and twist the tip of the electrode while cleaning.

Figure 39 - a clean stimulator

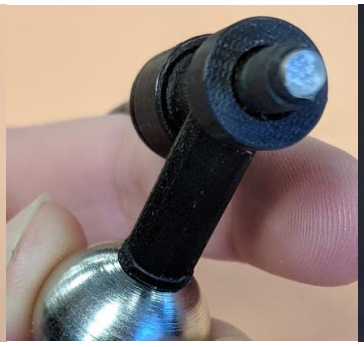


Figure 40 - a dirty stimulator

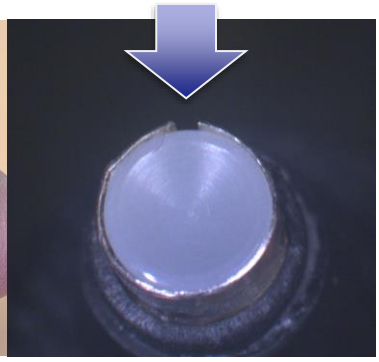


Figure 41 - a stimulator that has been damaged during cleaning

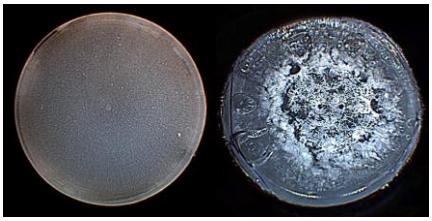


Figure 42 - Closeups of clean (left) and dirty (right) full-field electrodes

Cleaning Pattern Stimulator Electrodes

The cone of the pattern stimulator is very delicate, and care must be taken when cleaning! Place the tip of the stimulator under warm running water to loosen the hypromellose solution. An alcohol wipe, Kimwipe, or moistened cotton-tipped applicator may be used with care to remove the solution from the cup of the electrode. Use a twisting, not circular motion to remove solution from the tip of the stimulator.

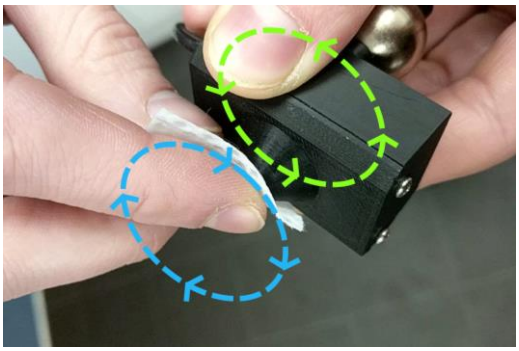


Figure 43 - cleaning the PERG stimulator

Local Configuration Settings

Configure System, accessible from the launch page of the software, permits operators to customize the configuration of their Celeris system. Most of the **Configure System** parameters are factory settings, and users should not change settings they are unsure of. Local configuration settings are stored in the **ESLOCAL.INI** file in the Celeris software directory, and Diagnosys recommends making a backup of this file before making any changes to Configure System parameters.

Examiner names

Examiner names can be added or modified in the Configure System > Program > Global section.

Institution name/address

The name and address of your lab can be added to your software. This will display on the launch page of the software and be added to any printouts. These parameters can be found under Configure System > Program > Global and are titled **Hospital Name** and **Hospital Address**.

Subject Identifiers

Subject Identifiers such as Animal Number, Cage number, Study ID, Date of Birth, Gender, etc. can be modified under Configure System > Program > Database under the parameter **Subject Field Names**. The related parameter **Subject Field Codes** controls whether these fields are visible and optional (code 1), mandatory (code 2), or suppressed (code 3).

The default (human) list of codes is: Family Name; First Name; Initial; DOB; |Hospital #; Gender; Referring Doctor; Ethnic Origin; Category; A Normal; Street; Town; State; Zip Code; Country; Telephone; OD Acuity; OD Refraction (SE); OS Acuity; OS Refraction (SE); Comment; Eye Color; User Field 1; User Field 2; User Field 3; User Field 4; User Field 5; User Field 6; User Field 7; User Field 8; User Field 9. Many of these fields are suppressed in the Celeris configuration. The underlined list of codes are visible from the subject selection page, and will be the most useful for the majority of users.

Subject Categories

Subjects can be assigned to and filtered by Categories. Examples of common categories include studies, investigators, or strains/models. The default list of subject categories can be found in Configure System > Program > Database > Subject Category. Once subjects are assigned to categories in the subject details page, operators can filter the Subject Selection Page by Category, so only patients assigned to a particular study/belonging to a specific investigator/of a particular model or strain are visible and can be selected at a time. Tip: keep categories YESTERDAY and TODAY – those will filter the subject view to only those patients tested on that date!

Backup/Autobackup

Diagnosys has two different backup routines. The first a serial backup, which can be set to remind you on a monthly/weekly/per session to make a dated copy of your database. The parameters for the default backup reminder interval and default backup location are found under Configure System > Program > Database under the settings **Backup Frequency** and **Backup Destination**. To disable this reminder, set the frequency to Manual. **DIAGNOSYS DOES NOT RECOMMEND DISABLING THE BACKUP REMINDER.**

The second routine is the Autobackup. At the end of each session where a change is made to the database, the software will place a copy of that database, the local configuration settings, and any defined calibration files (e.g. for the pattern stimulator) in a specified location. The autobackup can be toggled and the location set under Configure System > Program > Global with the parameters **Autobackup Enabled** and **Autobackup Directory**. Diagnosys **strongly recommends** leaving the autobackup enabled and setting the directory to a location not on the computer's hard drive. External hard drives or USB keys are highly recommended.

Control Bar Buttons

The color of the Control Bar buttons can be customized under Configure System > TESTMETHOD > [Test Method] > Display Control Button Colors. There should be three colors in the list; the first color indicates available commands; the second indicates the active command; the third indicates unavailable commands. The items in the list can all the be same color if desired. Note that this change may need to be made to both the ERG Test testmethod and VEP and ERG testmethod.

Display New Result Color

The default result colors can be customized under Configure System > TESTMETHOD > [Test Method] > Display New Result Color. This list can be considered circular – if you were to take 50 results in one step, it would loop through this list multiple times – and the item left selected is considered the start of the list, even if it is not the first item on the dropdown.

Tips and Tricks

Marker Placement

Diagnosys-supplied protocols will place markers automatically, but in some cases the marker placement may need to be corrected. To move a marker, click the **Scaling and Markers** button from the navigation bar and turn on the **Cursors On** button to enable the crosshairs. Ensure that you have the marker you'd like to move selected from the dropdown, then doubleclick the spot on the waveform you would like to move the marker. For more precise placement, you can use the Cursor → and ← Cursor buttons to move the cursor one time-unit at a time, and the **Place Marker** button to manually place the marker. *Tip: Right-clicking with the mouse will automatically select the next marker in the list!* If your animal has an extinguished response and you would like to remove markers from the waveform/marker table, the **All Markers On** / **All Markers Off** buttons will toggle marker visibility.

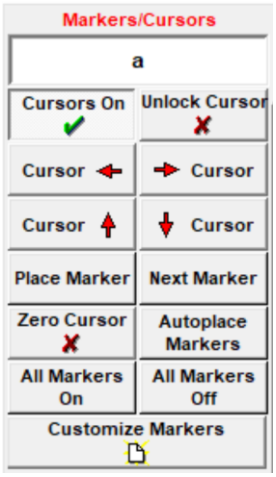


Figure 44 - marker placement

All Channels

At the bottom of the popout menu is a checkbox labeled **All Channels** that is enabled by default. This checkbox ensures that whatever change is made – deleting results, rejecting artifacts, or adjusting scaling – happens to all channels. If you would like to e.g. reject an artifact from only one graph, de-selecting **All Channels** before making the change will ensure that the change is only made to the selected channel.

Adjusting marker placement in protocol

If the marker placement frequently requires correction, the marker placement algorithm in the protocol may need to be adjusted. From the protocol selection page, select the protocol, press **Modify** and then press **Parameters**. Select the step with the faulty marker placement algorithm, then press the **Markers** button at top. Select the marker you would like to update at the lower left, then adjust the **Placement mode**, **Peak Start time (ms)**, and **Peak range time (ms)** as necessary. In Figure 45 at right, the software would attempt to place the marker at the lowest point between 0 and 40 ms.

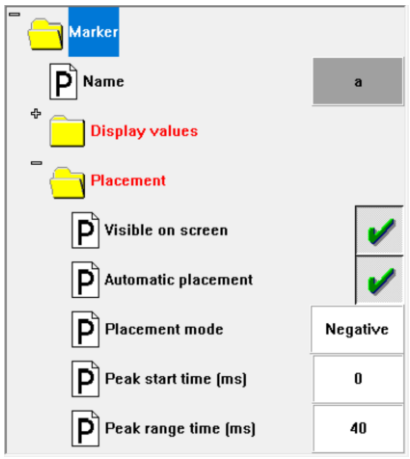


Figure 45 - marker placement settings

Networking Databases

There are several reasons you might want to put your Celeris database on your network:

- to allow multiple Diagnosys systems to share a single database
- to take advantage of your server's automatic backup features
- to allow an analysis machine in your office to connect to the system's database, so you can access the database and analyze tests from the comfort of your office

Instructions for how to network your database can be found on the Diagnosys website.

Reassigning a test to the correct subject

In the event that a test is performed under the wrong subject, reassigning the test is simple! From the subject selection page, locate the correct (destination) subject and note down the subject's **record number**. Then, locate the subject the test was recorded under and press **Old Tests**. Select the test you would like to reassign and press the **Comment** button at the lower left. A test details page will appear with an **Assigned to Patient** checkbox. Toggle the checkbox to enable the command and change the record number to that of the destination patient. Close the details page and save changes to commit the reassignment.

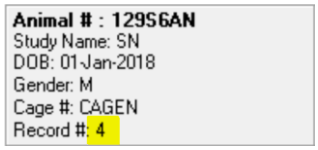


Figure 46 - record number

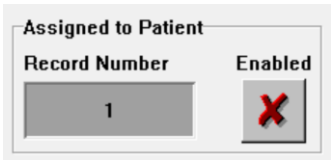


Figure 47 - assigned to patient

Export Lists of Patients/Tests

You can export lists of tests or subjects tested. From the subject selection page, selecting a subject category, or typing in two dates in MM/DD/YYYY MM/DD/YYYY format will filter the list of patients to only those patients assigned to that category, or who had tests performed between those two dates. Once the subject list has been filtered to your liking, the Export button (indicated by the yellow box in Figure 48) will export a list of patient records in TXT or CSV format (see Figure 49). This can be a useful way to keep track of daily test progress.

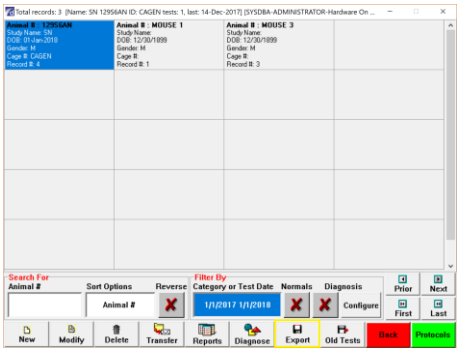


Figure 48 - filtered list of test subjects

Patient Record	Unique ID	Animal #	Study Name	Initial	DOB	Cage #	Gender	Investigator
4	918F1B49-BA9F-4CA6-9839-070B-50E8-84F7	129S6AN	SN		1/1/2018	CAGEN	Male	
1	97BA9A9B-58B8-43AD-B879-D3B5-EA85-EA8E	MOUSE 1			12/30/1899		Male	
3	F301C139-A554-4137-AB69-D2B1-802F-5603	MOUSE 3			12/30/1899		Male	

Figure 49 - exported list of patients