

b. ZEUS ELECTRO DISH

Electro Dish(ED) is a specially designed disposable sterile petri dish with metal electrodes and four separate pools between which the cells are placed (Figure 6-A). There are points of electrode cables connection coming out of the device between the two bridges at the front of the petri dish (Figure 6-B).

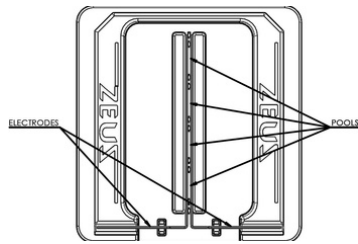


Figure 6 - A

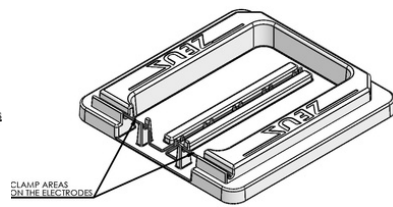


Figure 6 - B

c. ZEUS DISH FASTENER

Dish Fastener (DF) is a metal block designed to fit in the channels on the sides of the petri dish (ZEUS Electro Dish) and made for keeping the petri dish stable during processing. It also acts as a thermal block. Due to its U-shaped structure, it enables the petri dish to be viewed under a stereo microscope (Figure 7).

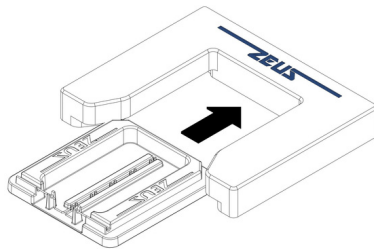


Figure 7

d. ZEUS ELECTROLIQUID

Electroliiquid Solution(ES) is a sterile electrolyte solution prepared to transmit electric current to the cell. It contains Mannitol solution, MgSO₄, CaCl₂, HSA and HEPES. Electroliiquid vials should be stored at 2-8 ° C. Electrolyte concentrations in this product are very sensitive and it is stable until the expiration date shown on the vial label.

e. ZEUS ELECTROLIQUID PREPARATION & PIEZOELECTRIC APPLICATION

i. APPLICATION PROTOCOL

1. Prior to use, Electroliiquid Solution (ES) should be heated up to 37° for a minimum of 30 minutes.
2. The channels on Electro Dish (ED) should be filled with 30 µl heated ES with a micropipette.
3. As shown on Figure 8, on the ED 4 40-µl ES washing droplets are made.
4. 20-30 minutes after ICSI procedure, the oocytes are taken from the culture medium, first they are transported to the first wash droplet and washed by pipetting, and then they are washed by pipetting at the 2nd,3rd and 4th droplets. Ensure that oocytes are always on the droplet floor while washing.
5. After washing (gradual adaptation) oocytes are placed in the pools filled with ES solution (Figure 8). Cells should be placed in the midline of the channel within equal distance from the electrode rods. Do not add mineral oil (Figure 8/3-A).

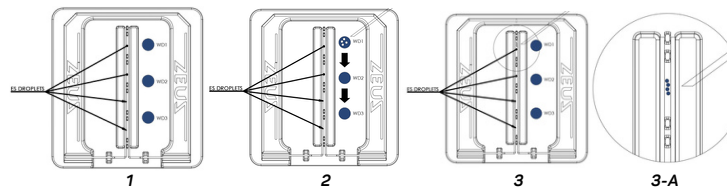


Figure 8

6. After placing the cells in the channels, ED is placed on an inverted or stereo microscope and fixed by means of "Dish Fastener" (DF). Then, using clamps the connection is made to the appropriate electrode poles (Figure 6-B).
7. During the procedure, continue following the cells under an inverted or stereo microscope. Apply electrical stimulation according to Device Usage Instructions within Application Instructions. At this stage, Dish Fastener can be used to fix the petri dish (ED) under a microscope (Figure 7).
8. Immediately after electrostimulation, aspirate the oocytes from the electrode channels, wash them in culture medium, and then transfer them to a standard culture dish to follow the conventional embryo culture procedure.

ii. ELECTROLIQUID STORAGE CONDITIONS AND STABILITY

Electroliiquid Solution (ES) vial at 2-8 ° C. This product is stable until the expiration date shown on the label on the vial.

iii. MATTERS TO TAKE INTO CONSIDERATION

- Do not resterilize, do not reuse.
- Do not use blurred or yellowed solutions.
- If you notice anything unusual about the features on the label, do not use the product.
- Do not use if the sterile ED package or ES vial has been opened or damaged.
- Products should be stored in their original unopened packaging at 2-8° C after delivery.
- Do not use the product after expiration date.
- Aseptic techniques should be used during procedures.
- Use only sterilized equipment and materials.
- Follow all local environmental regulations when disposing.
- Read the instructions for use before use.

iv. OPERATING THE DEVICE (Electrical Stimulation)

Connect the device to an earthed wall socket using the provided adapter (adapter input), and connect the electrode cable to the electrode input. After the connections are made, you can operate the device from the ON / OFF button. When the device is turned on, first the opening screen and then the application screen will appear (Figure 2 and 3). As of this step, follow the directions at the bottom of the screen, first select the type of impulse (DC or AC / DC mode), then follow the directions and perform the application with the LOAD and then APPLY buttons. At any point, press the BACK button to return to the previous screen.



USER MANUAL

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1 DEVICE NAME & DESCRIPTION

- Device Name** : ZEUS Pulse Stimulator
Device Description : Manufacturer of cell specific constant direct current (DC) and alternating current (AC).
- a. Authorized Company** : ZEUS Bioscience Biyomedikal A.Ş.
b. Producing Company : GenSa Genel Sağlık Hizmetleri A.Ş.
c. Brand – Model : ZEUS Pulse Stimulator - ZPS 24

2 GENERAL WARNINGS

Read the User Manual carefully before using the device and take into consideration the indicated warnings.

This manual has been prepared for the safe, correct and productive use of the device you have received. The information and explanations that you will need to use the device are included in the manual. For the issues that cannot be solved you can contact the authorized company.

Do not place or use your device on wet / damp surfaces. Use a grounded outlet when using.

3 INTRODUCTION & GENERAL INFORMATION



a. INTENDED USE & PRINCIPLES OF OPERATION

ZEUS Pulse Stimulator provides electroporation and fusion of the cell with suitable electrical current. The device can offer alternating current (AC) and direct current (DC).

b. TECHNICAL SPECIFICATIONS

The device is controlled by touch-screen and designed to prevent the errors that may arise from the user or the network by generating fixed and standard stimulus at one time with the command, first storing the current so that it is not affected by the city network and voltage fluctuations. ZEUS Pulse Stimulator is designed for cell suspension and in vitro applications. The device produces stable, standard and safe pulses at the cellular level only with its specific apparatus (3-c.). Technical data of the device is as follows:

Voltage / Amps	: 24 V / 3 A
Stimulus Duration	:
AC	: Adjustable
DC	: Adjustable
Device Dimensions	: 140 x 240 x 190 mm
Weight	: 3.425 g
Control Panel	: 4.3 inch touchscreen

c. COMPONENTS & UNITS

ON / OFF (Power) button, digital touch screen, electrical input, electrode outputs are located on the device. Power cable, electrode cable and tips, disposable sterile dish (electro dish), dish fastener, sterile non-toxic electrolyte (electrolyte), main body, user manual, warranty certificate are included with the device package.

4 INSTALLATION & USAGE

The device will be delivered to the user in a shielded original box. The device and the accompanying apparatus should be compared with the list of box contents in the user manual to check for any missing parts. The user manual must be read carefully before installing and using the device. Device installation and start-up is done by the user. The device must be placed on a

suitable worktop and never on wet or damp surfaces. The device should be connected to a grounded outlet. The device is ready for usage after turning ON / OFF button. The type of current (AC / DC) is selected from the options on the touch screen and by following the commands on the screen the impulse is given to the cell which was previously prepared between the electrodes in the petri dish. The related details are provided in the section "10. APPLICATION INSTRUCTION". Direct technical assistance (English / Turkish / Russian) can be provided during installation if necessary.

5 SHIPPING

The device is shipped within 5 days after payment. Shipping costs are paid by the user. Transport of the device will be done through licensed and accredited cargo companies. Regarding the issues that may occur during transportation the warranty and operation protocols of the transportation company shall be valid. Upon the request the device can be collected directly by the receiver.

6 FAULT FINDING & REMOVAL

Authorization of sale of ZEUS Pulse Stimulator device is given by the letter of 89252994-401.01.05 dated 17/09/2019 of Directorate General of Consumer Protection and Market Surveillance of the Ministry of Trade. The warranty certificate has been prepared in accordance with the "After Sales Services" regulation prepared by the Ministry of Trade based on Article 58 of the Law No. 6502 on Consumer Protection and published in the Official Gazette No. 29029 dated 13.06.2014. In case of malfunction of the device, please contact the vendor. In the event of warranty coverage, the existing defective device shall be replaced by a new device. A replacement device will be shipped to your address within one week of receiving the returned device.

7 SECURITY RECOMMENDATIONS

The user manual should be read carefully before using the device. The device should not be stored and used on wet and damp surfaces. The device should be stored and used on a suitable table or worktop.

Since the device has an electrically operated system, it should be used only by authorized persons with the necessary general safety precautions.



8 WARRANTY

The device is not covered by the warranty in case it was misused or used for other purposes. The user must check that all apparatus and parts are complete before installation. The user accepts that the manufacturer is not responsible for any damages, accidents, etc. that may occur during the installation and usage of the device. The buyer accepts these warranty terms when purchasing the product. Details of the warranty conditions are stated in the warranty certificate which is delivered to the buyer after the device is approved by the seller.

9 DEVICE CONTENT

- 1 x Pulse Stimulator - Main Body
- 1 x Adaptor
- 2 x Disposable Sterile Dish (Electro Dish - ED)
- 2 x Electrolyte Solution (Electrolyte Solution - ES)
- 1 x Crocodile Cable

- 1 x User Manual
- 1 x Warranty Certificate
- 1 x Electro Dish Fastener (Dish Fastener - DF)
- 1 x Transport Bag

10 APPLICATION INSTRUCTION

a. ZEUS ZPS24 DEVICE INSTRUCTION MANUAL

Connect the device to a wall socket with the "adapter input socket" (power input) using the provided adapter, and connect the electrode cable to the crocodile cable input. After the connections are made, the device can be operated with the ON / OFF button. (Figure 1).



Figure 1

When the device is turned on by the ON / OFF button, first the opening screen will appear (Figure 2), then the application screen with three touch buttons will be displayed where the application mode can be selected (Figure 3). On the screen there are "DC Mode" button that gives direct current and "AC Mode" that gives alternative current, as well as "Settings" button used to adjust the current time. When the preferred current type is selected, the third screen will appear with "Load" and "Apply" and "Back" buttons (Figure 4). To apply the selected current, it is sufficient to follow the "Instruction" menu at the bottom of the screen. When the "Load" button is pressed, the device will be ready to give current and when the "Apply" button is pressed, the device will provide impulses to the electrodes on the electro dish. Before the impulse is given, the electrode preparation must be completed and the cells must be placed in the relevant section (see ZEUS ELECTRO DISH / ZEUS ELECTROLIQUID PREPARATION). The factory default setup settings of the device are set to 70 µs for the option of DC Mode and 5 seconds for the AC / DC Mode option. When required, the time can be set differently via "Settings" button (Figure 5). During stimulation, the device's functionality can be verified by observing the LED indicator. The LED indicator apparatus is available as an optional accessory.



Figure 2

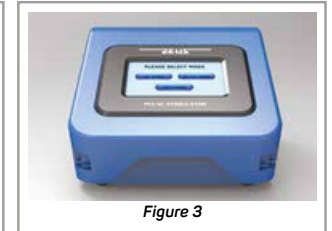


Figure 3



Figure 4

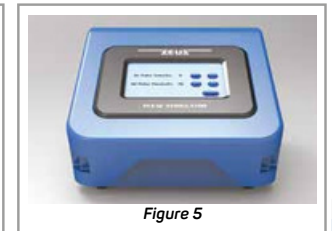


Figure 5