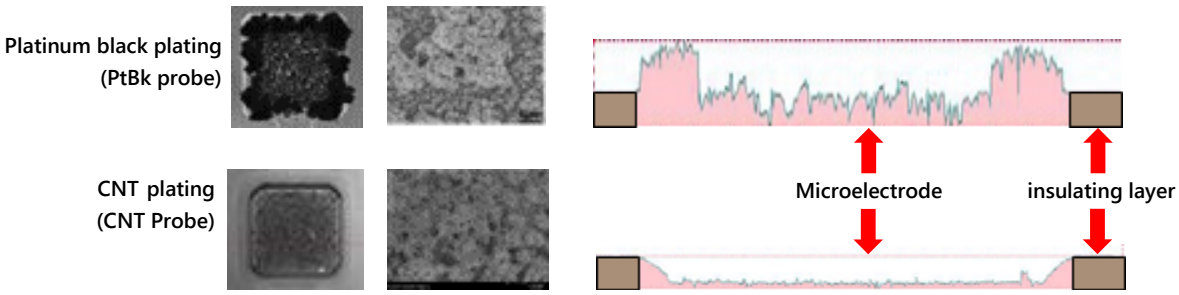
MED Probe

User's manual

1. Introduction

The MED probe is an electrophysiological probe enable researchers to measure the electrical activity of excitable cells "at multiple points, simultaneously, and over a long period of time." It is applied to preparations such as acute brain slices, dispersed culture systems, and organ culture systems. The MED64 system can not only detects the erectrical potential between the four reference electrodes and each of the 64 recording electrodes, but also deliver electrical current to any recording electrode. The electrodes are plated with black platinum or carbon nanotubes (CNT) on an ITO lead pattern to expand the effective electrode surface area to 100-200 times, resulting in an improved signal-to-noise ratio and the ability to apply high-intensity currents. As for the MED probe with CNT-plated electrodes (hereinafter referred to as CNT probe), it achieves "improved physical durability" and "flat electrode surface structure" while maintaining the same electrode performance as the original MED probe with platinum black-plated electrodes.



2. How to use

2.1. Opening the package

The MED probe is packaged in a non-sterile, pre-cleaned condition. If there is any dust on the surface after opening, wash off them with distilled water using a wash bottle.

Note: Do not touch the electrode surface of the MED probe during cleaning. Doing so may damage the electrode and insulation layer.

2.1.1. Approximate storage limitation in unused condition

Even if the MED probe is brand-new, its electrode performance will deteriorate over time if it is not used for a long time. When storing MED probes for a long time in unused condition, open the package within 3 months and keep the chamber filled with double distilled water (hereinafter referred to as DDW) or sterile distilled water (hereinafter referred to as SDW) until the time of use.

2.1.2. Expiration date and frequency of use

We do not guarantee the performance of the MED probe that have been manufactured for more than one year, regardless of the storage conditions. Although the MED probe can be reused with proper handling, we do not guarantee the performance of the reused probes since they are designed to be disposable.

> *Replacement policy*
> Incase of quality issue of a brand-new MED probe, we will replace them if it is within half a year from the purchase.

2.2. Sterilizing the MED probe

When culturing cells on the MED probe, the following sterilization procedures should be carried out as necessary after opening the package.

(1) Immerse the MED probe in 70% ethanol for 15 minutes.

Note: Do not leave it in 70% ethanol for more than 30 minutes to prevent it from affecting the insulation layer, etc.

(2) After drying it naturally in a clean bench, irradiate with a UV lamp for 15 minutes.

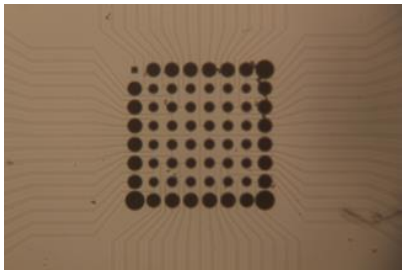
Note: Do not autoclave it. Autoclave may destroy the electrodes.

2.3. Hydrophilize the surface of the MED probe

Since the electrode surface of an unused MED probe is relatively hydrophobic, hydrophilization before use is needed. At this time, not only treat the electrode surface for a predetermined period of time, but also confirm the surface visually during rinsing to check if it has changed to a moist and wet state without water droplet. Note that this procedure is not always necessary for reuse of the MED probe, since it will gradually become hydrophilic with repeated use and proper storage. Typical procedural examples are shown below.

2.3.1. Polyethyleneimine

(1) Fill the MED probe with 0.1% polyethyleneimine (PEI) solution dissolved in 25 mM borate buffer and incubate at room temperature overnight. The PEI solution may cover the electrode with air bubbles, in that case, remove the air bubbles by pipetting.



(2) Rinse over three times with DDW or SDW before use.

How to prepare 0.1% polyethyleneimine solution

(1) Preparing borate buffer solution.
❶ Dissolve 9.525 g of sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$) in 950 ml of distilled water. ❷ Adjust the pH to 8.4 with 1N-HCl. ❸ Add DDW up to 1000 ml. ❹ Store it under 4°C.

(2) Preparing 1% polyethyleneimine solution.
❶ Because polyethylenimine is originally a 50% solution, weight and dispense it into a centrifuge tube (by using a pipettor with a 1 ml tip cut off), and then dilute 5 times with borate buffer. ❷ Further dilute 10 times to adjust to 1% solution. ❸ Store it in a refrigerater.

(3) At the time of use, further dilute with borate buffer solution to adjust to 0.1% solution.

2.3.2. Serum

Fill a new-new MED probe with serum (used medium containing serum for cell culture is also acceptable), remove it after 1-2 minutes, and rinse it three times with SDW.

2.4. Coating the surface of the MED probe with extracellular matrix

In the case of culturing cells on the MED probe, coat the hydrophilized surface with extracellular matrix prior to seeding cells. Since the affinity with the extracellular matrix may be different depending on the cell type, strain or line, coat an appropriate extracellular matrix based on the basis of your experience. Typical examples of coating are shown below.

2.4.1. Poly-D-lysine [for primary neurons]

(1) Dissolve poly-D-lysine (Sigma P6407) in SDW to a concentration of 50 µg/ml.

(2) Pour 1 ml of poly-D-lysine solution into the MED probe to cover the entire bottom surface, and then leave it in the incubator for at least an hour.

(3) Remove as much of the poly-D-lysine as possible by using a pipette, and then rinse three times with SDW. The removed poly-D-lysine may be reused for coating other MED probes.

2.4.2. Fibronectin [for human iPS cell-derived cardiomyocytes]

(1) Drop 2 µl of 50 µg/ml fibronectin solution onto the recording electrodes and leave it in the incubator for an hour. To prevent the fibronectin solution from drying out, place the MED probe in a Petri dish and incubate it with about 3 ml of

SDW around it.

(2) Immediately before seeding cells, aspirate the cell suspension with an aspirator (or do not aspirate) so that a small amount of fibronectin solution remains on the electrode area, and then quickly seed the cell suspension.

2.4.3. Matrigel [for human iPS cell-derived cardiomyocytes].

(1) Drop 2 µl of Matrigel (BD Biosciences #354277) solution onto the recording electrodes only. Regarding the Matrigel solution, prepare according to the manufacturer's instructions, dispens and store at -20°C, and dissolved immediately prior to use.

(2) Place the Matrigel-coated MED probe in an incubator for at least 30 minutes.

2.4.4. Collagen [for slice culture]

All procedures should be performed under sterile conditions. To ensure that there is no contamination of the coated MED probe, this protocol should be performed at least 8 hours before the start of incubation.

(1) Sterilize the MED probe with 70% ethanol.

(2) Dry the MED probe naturally in a clean bench, and then sterilize it by UV irradiation for 15 minutes.

(3) Place the MED probe into the refrigerator for at least one hour to chill thoroughly.

(4) Retrieve the MED probe from the refrigerator and pour collagen solution into the chamber to completely cover the surface of the MED probe. Then, immediately remove as much of the collagen solution as possible using a pipette, making sure that the surface of the MED probe is evenly wetted with the collagen solution. The removed collagen solution may be reused to coat other MED probes.

(5) Incubate the MED probe in a CO_2 incubator (37°C) for 30 minutes to allow the collagen to gelate.

(6) Rinsing the surface with SDW three times.

(7) Pour medium into the chamber and store in a CO_2 incubator until just before use (up to a week).

How to prepare collagen solution (Nitta gelatin; Cellmatrix Type I-A)

Preparation should be performed under sterile conditions, at 4°C.

(1) Add 1 ml of 10-fold DMEM/F-12 mixture to the collagen gel solution (0.01N HCl, pH 3.0), and then mix them gently.

(2) Add reconstitution buffer (0.08N sodium hydroxide, 200 mM HEPES) to the above mixture, and mix them gently.

(3) Store the final mixture in a refrigerator for 30 minutes to remove any air bubbles (this step may be omitted).

2.5. How to clean after use

2.5.1. For acute brain slice

(1) After removing the anchor, fill the MED probe with water or artificial cerebrospinal flood. Shake it gently from side to side to float the slice, and then scoop it out with a paintbrush.

(2) Rinse two or three times with DDW.

2.5.2. Trypsin EDTA

(1) Remove the culture medium, fill the chamber with trypsin EDTA immediately afterwards. Incubate it at 37°C for about an hour.

(2) Detach the cells by pipetting several times.

(3) Remove the trypsin EDTA by using an aspirator, and then wash two or three times with SDW.

2.5.3. Combination of TrypLE Express and Tergazyme

(1) Aspirate completely whole medium from the MED probe. Immediately add 300 µL of TrypLE Express Enzyme (Gibco, Cat# 12604013) into the chamber.

(2) Incubate the MED probe at 37°C for 1 hour.

(3) Take the MED probe out of the incubator. Pipette deliberately and carefully at the chamber to detach cells by water pressure.

Note: Be extremely careful not to damage the electrodes.

(4) Aspirate the TrypLE Express completely from the chamber.

(5) Rinse the MED probe with PBS at least 3 times.

(6) Fill the chamber with 300 µL of 1% Tergazyme® solution.

Note: Dilute Tergazyme® with sterilized water, and then warm it up (55°C) to dilute completely.

Note: Prepare the Teragazyme® solution BEFORE used.

(7) Leave the MED probe at room temperature for 1 hour.

(8) Pipette again deliberately and carefully at the chamber.

(9) Aspirate the Tergazyme® solution from the chamber. Rinse the MED probe with SDW at least 3 times.

Note: If the MED probe is not cleaned well enough, repeat #3-8.

(10) Fill the chamber with 70% ethanol. Leave it at room temperature for 15 minutes.

Note: Avoid exposing to ethanol for longer than 15 minutes.

(11) Rinse the MED probe with SDW at least 3 times.

(12) Store the MED probe with electrodes moisten (in SDW) at a refrigerator (4°C)

2.5.4. Cleaning with bleach

Note: Unlike the platinum-black probe, removal of cell residues with chlorine bleach is not possible with the CNT probe, as it may damage an electrode.

(1) With cultured slices or cells present, rinse the chamber three times with approximately 1 ml of bleach (such as Clorox).

(2) Pour the bleach into the MED probe and leave it for 15-30 seconds.

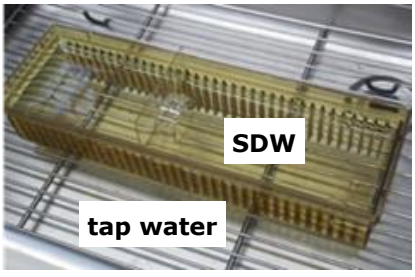
Note: If the probe is not fully cleaned after this treatment, rinse it twice more and then expose the MED probe to the bleach for a long time. Normally, one or two minutes is sufficient, but up to 15 minutes is possible. Also, if cells are dead and stuck to the electrodes for a long time, the cells may peel off the electrodes. Therefore it must be performed as soon as possible after the experiment.

(3) Remove the bleach and rinse the MED probe at least five times with DDW.

2.5.5. Ultrasonic cleaning

If the CNT probe is still noticeably contaminated after the trypsin treatment described above, ultrasonic cleaning may be performed if necessary. Be sure to place the MED probe in a plastic container filled with DDW or SDW for indirect cleaning. Washing directly in a tank or indirectly in a glass container such as a beaker may damage the electrode. The washing time should be kept within 3 minutes per washing. Do not perform the procedure when the cells are stuck to the surface.

Note: Do not perform this procedure in a PtBk probe because it may damage the electrode.



2.6. How to store after use

The impedance of the MED probe may increase when the electrodes dry out after use, and the original low impedance cannot be maintained. Be sure to store it properly according to A or B below.

(A) Place the MED probe in a beaker or other container containing DDW (preferably SDW) and store it in a refrigerater.

(B) Pour DDW (preferably SDW) in the chamber, seal it with Parafilm, and store it in a refrigerater.

Note: In either case, replace the DDW or SDW at least once a month for long term storage.

2.7. Other precautions

2.7.1. About the insulating layer

The surface of the MED probe is covered with an insulating film except for the electrode surface. Please be careful not to touch it with tweezers or the tip of a micropipette because it is a very delicate material like the electrode surface.

2.7.2. Stimulation current applied to the electrode

Use the settings within the red area of the graph of stimulation current and application time below. Use of settings outside this range may cause damage to the microelectrode or measurement sample (Figure 1).

2.8.3. Handling of CNT probes filled with culture medium

If the CNT probe is left in the air (outside of a 5% CO_2 environment) while filled with culture medium, the impedance will increase significantly in about two or three hours, resulting in a significant decrease in electrode performance (Figure 2). To avoid this, the MED probe should be returned to the incubator or cleaned immediately after use. If the performance of the MED probe is degraded by leaving it in the air, it can be restored to its original performance by sonicating it for 3 minutes and then storing it in DDW for at least 12 hours. For more information on ultrasonic cleaning, please refer to p.4 "2.5.4. Ultrasonic Cleaning".

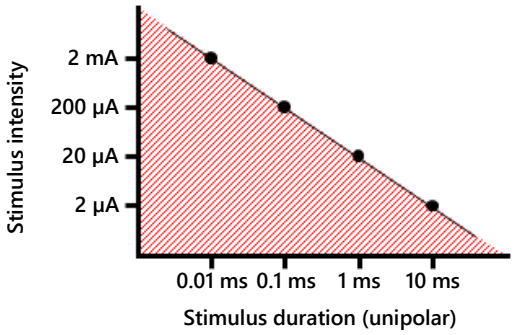


Figure 1

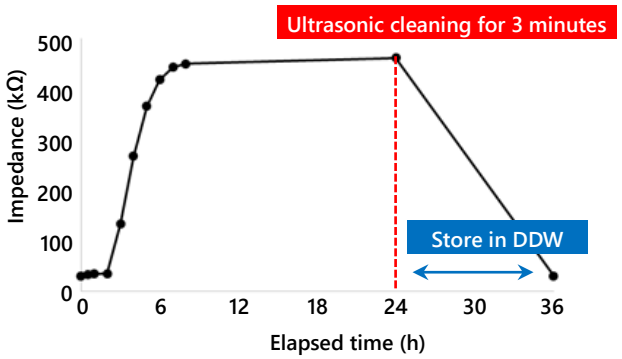


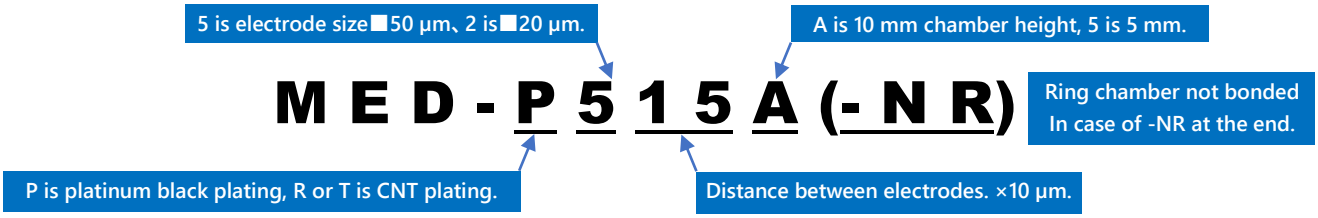
Figure 2

2.8.4. Boiling disinfection

The MED probe can be disinfected by boiling. The electrode surface should be placed face up, as air bubbles from the bottom of the boiler (or pot) can damage the electrode if it is boiled face down. Start boiling with water, bring to a boil, and boil for up to 20 minutes. After boiling, avoid exposing the glass substrate to water to prevent it from breaking, and let it cool down naturally.

2.8.5. The definition about the probe number

Except for some special product numbers, it is defined by the following rules.



3. Ratings and specifications

3.1. General

Glass substrate	
Material	glass
Dimensions: MED Probe	Length 50 x Width 50 x Height 0.7 mm
MED Probe 16	Length 40 x Width 30 x Height 0.7 mm
MED Multiwell Probe	Length 35 x Width 100 x Height 0.7 mm
Conductive layer material	Indium Tin Oxide (ITO) [0.15 μm thick]
Insulating layer material ※	Polyimide [1.5 μm thick] or acrylic resin [1.5 μm thick]
Chamber	
Material: MED Probe	Glass or acrylic resin
MED Probe 16	glass
MED Multiwell Probe	acrylic resin
Dimensions: MED Probe	Outer diameter 25 mm, inner diameter 22 mm, height 10 mm (MED-P###A) Outer diameter 25 mm, inner diameter 22 mm, height 5 mm (MED-P###5)
MED Probe 16	Outer diameter 25 mm, inner diameter 22 mm, height 10 mm
MED Multiwell Probe	Well bore diameter Φ16 mm, well spacing 18 mm, height 10 mm (MED-#5NF30) Well bore diameter 7.5 x 16 mm, well spacing 9 mm, height 10 mm (MED-#5N811)
Recording electrode	
Material	Platinum black or carbon nanotubes
Number of Electrodes	64
Dimensions	50×50 μm (MED-#5###) / 20×20 μm (MED-#210#) / Φ50 μm (MED-#5001#、MED-#5002#、MED-#5003#) / Φ20 μm (MED-#2H08#)
Impedance (1 kHz, 50 mV sinusoidal wave applied)	< 22 kΩ (MED-#5###) / <30 kΩ (MED-#210#)
Maximum allowable current	±200 μA (0.1 ms wide)
Maximum allowable voltage	±1 V (0.1 ms wide)
Reference electrode	
Material	Black platinum or carbon nanotubes (CNT)
Number of Electrodes	64
Dimensions	50×50 μm (MED-#5###) / 20×20 μm (MED-#210#)
Impedance (1 kHz, 50 mV sine wave applied)	< 2.2 kΩ

※Specification chang on the insulating layer material

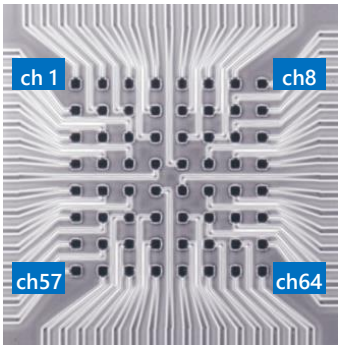
Since March 2017, we have been changing the insulating layer material into polyimide as soon as we run out of stock of glass substrates with insulating layer made of acrylic resin. The product numbers with the insulating layer changed as of February 2020 are as follows.

MED-#210#, MED-#515#, MED-#530#, MED-#545#, MED-#G515#

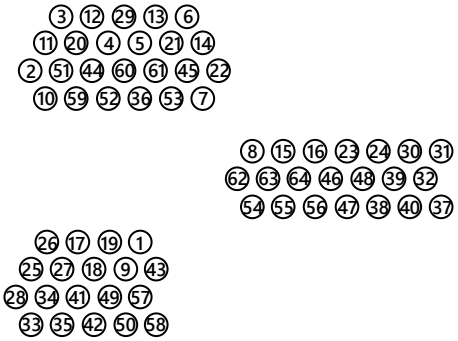
3.2. Electrode configuration

3.2.1. Standard 8 x 8 arrangement

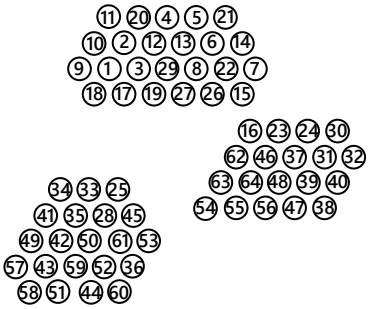
(electrode number with the output terminal of the MED connector positioned on the right side)



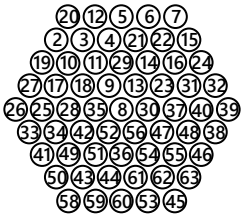
3.2.2. Special arrangement (electrode number with the output terminal of the MED connector positioned on the right side)



Array for rat hippocampal slice (MED-#5001#)



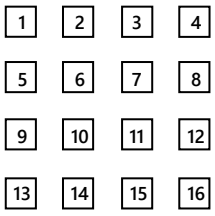
Array for mouse hippocampal slices (MED-#5002#)



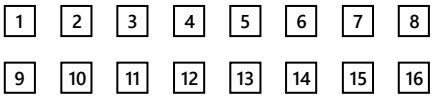
hexagonal array
(MED-#2H07#)

Note: In hexagonal arrays, signals can not be recorded on ch1, ch57 and ch64 where no electrodes are placed. In order to reduce the size of the data file, it is recommended to turn off ch1, ch57 and ch64 in the Channel setting field of the Acquire MED64R2 data module in Mobius.

3.2.3. MED Probe 16 (electrode number with the terminals located on top

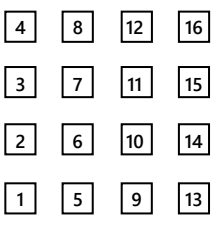


MED-#G515A

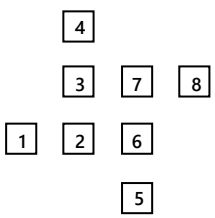


MED-#G501A

3.2.4. MED Multiwell Probe (electrode number with the terminal located on top)



MED-#5NF30



MED-#5N811

Version: 211105

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