Mini Vertical Electrophoresis Cell

L-VET-MINI4 Operations Manual

L-VET-MINI4 — 2023.4 Edition

Contents

Important Safety Information

This manual contains important operating and safety information. To use this instrument correctly and safely, please read and understand this manual before operation.

The L-VET-MINI4 has been designed and manufactured to meet recognised safety standards; operating it strictly in accordance with these instructions will be safe. The instrument must not be modified or altered in any way.

The upper lid is part of the safety interlock: when the lid is opened, the electrical circuit is automatically cut off. Never attempt to operate the cell without the upper lid in place.

Do not expose any component of the cell to acetone or ethanol. Damage caused by the use of organic reagents is not covered by the warranty.

Note: The company is not responsible for any consequences arising from use that does not follow this manual.

Chapter 1 Product Introduction

1. Overview

The L-VET-MINI4 mini vertical electrophoresis cell runs both precast and hand-cast gels, with up to 4 gels running simultaneously, and is compatible with 1-D and 2-D electrophoresis applications. The casting frame, together with the spacer glass plates with fixed side strips, makes hand-casting simple and helps prevent gel leakage.

2. Structure and Components

For best results, familiarise yourself with each component and with how the parts are assembled and disassembled (see Figures 1 and 2).

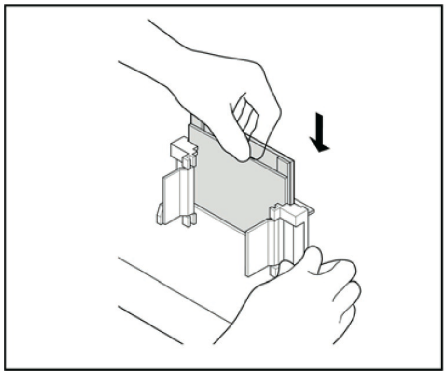

Figure 1: L-VET-MINI4 cell assembly

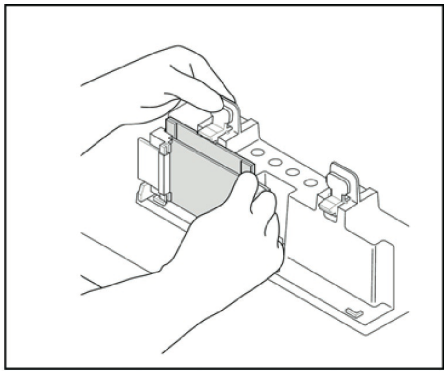

Figure 2: Assembly of the casting frame and casting stand

- Spacer glass plate (with fixed side strips): the taller glass plate fitted with fixed side strips. Side-strip thicknesses available: 0.75 mm, 1.0 mm and 1.5 mm.
- Plain glass plate: the shorter glass plate, which combines with the spacer plate to form the gel sandwich.
- Casting frame: placed on the bench to align the spacer plate and the plain plate and to hold them together as a gel sandwich.

- Gel cassette assembly: one casting frame, one spacer glass plate and one plain glass plate.
- Casting stand: the pressure lever seals the gel cassette against the casting gasket, ensuring the cassette does not leak during casting.
- Gel sandwich: the spacer plate, the plain plate and the polymerised gel between them.
- Single-gel replacement plate (buffer dam): a clear buffer dam used when running 1 or 3 gels.
- Electrode core: holds the gel sandwiches. Fitted with a U-shaped gasket and positive and negative electrodes with connector plugs. The positive electrode is marked red, the negative electrode black.
- Buffer chamber and upper lid: the buffer chamber and lid close to ensure proper electrophoresis; opening the lid cuts the circuit. The chamber and lid are also compatible with other electrophoresis modules, such as blotting/transfer, the first dimension of 2-D electrophoresis and electroelution.

Packing list and accessories

After purchasing the instrument and before use, check the contents against the list below and inspect each item for damage. Items showing a standard quantity are supplied with the cell in its standard configuration; the remaining items are compatible accessories and spare parts that may be ordered separately by part number.

Part No.	Name	Description	Pack unit	Std. qty
810103032	Precast-gel vertical electrophoresis cell	Upper shell (with cord), lower shell, plug-head electrode core, mushroom-head electrode core, buffer dam, 5 gel-prying spatulas	1 set	—
810103003	Comb	1.0 mm, 10 wells	5 pcs/pack	5
810103004	Comb	1.0 mm, 15 wells	5 pcs/pack	5
810103005	Comb	0.75 mm, 10 wells	5 pcs/pack	—
810103006	Comb	0.75 mm, 15 wells	5 pcs/pack	—
810103007	Comb	1.5 mm, 10 wells	5 pcs/pack	—
810103008	Comb	1.5 mm, 15 wells	5 pcs/pack	—
810103009	Spacer glass plate (with side strips)	0.75 mm, 101 × 82 mm	5 pcs/box	—
810103010	Spacer glass plate (with side strips)	1.0 mm, 101 × 82 mm	5 pcs/box	5
810103011	Spacer glass plate (with side strips)	1.5 mm, 101 × 82 mm	5 pcs/box	—
810103012	Plain glass plate	1.0 mm, 101 × 73 mm	10 pcs/box	10
810103013	Casting stand	100 × 73 mm, for gel casting	1 pc/pack	4

Part No.	Name	Description	Pack unit	Std. qty
810103014	Casting frame	For gel casting	1 pc/pack	4
810103024	Casting set	Casting stand + casting frame + 2 sets of 1 mm glass plates + 2 each of 1 mm 10-well and 15-well combs + 1 casting gasket	1 pc/pack	—
810103015	Casting-frame hinge	Mounted on the casting frame	2 pcs/pack	8
810103016	Casting gasket	Seals the glass plates; placed under the casting stand	5 pcs/pack	5
810103017	U-shaped gasket	Seals the gel chamber; mounted on both sides of the electrode core	2 pcs/pack	4
810103018	Electrode-core side clamp	Mounted on both sides of the electrode core for clamping	2 pcs/pack	4
810103019	Plug-head electrode core	Direct power connection	1 pc/pack	1
810103020	Mushroom-head electrode core	Indirect power connection	1 pc/pack	1
810103021	Buffer dam	Used when running a single gel	1 pc/pack	1
810103022	Gel-prying spatula	For prying the glass plates apart	5 pcs/pack	5
810103023	Loading stand	For convenient sample loading	1 pc/pack	1
810100002	Upper shell (with cord)	Upper lid with power cord	1 pc/pack	1
810100003	Lower shell	Buffer chamber	1 pc/pack	1
810100001	Electrophoresis leads	Universal for electrophoresis power supplies	1 pair/pack	—

Note: “Std. qty” is the quantity included in the standard configuration; “—” indicates the item is an optional accessory or spare part, not included as standard.

Maximum sample volume per well:

Wells	Width per well	0.75 mm	1.0 mm	1.5 mm
10	5.08 mm	33 µl	44 µl	66 µl
15	3.35 mm	20 µl	26 µl	40 µl

Chemical compatibility:

None of the components of the L-VET-MINI4 cell may come into contact with acetone or ethanol. Damage caused by the use of organic reagents is not covered by the warranty.

Avoid repeated contact with 100% TEMED. Prolonged, repeated rubbing with TEMED before casting will damage the structural integrity of the combs.

3. Main Technical Parameters

Parameter	Specification
Model	L-VET-MINI4
Type	Mini vertical electrophoresis cell (PAGE); 1-D and 2-D compatible
Gel format	Precast or hand-cast gels
Gel capacity	1–4 gels run simultaneously
Precast-gel compatibility	Compatible with PIERCE and BIO-RAD precast gels
Gel thickness	1.0 mm (standard); 0.75 mm, 1.5 mm
Glass plate size	100 × 83 mm
Gel size	83 × 73 mm
Comb specifications	1.0 mm thick, 10 / 15 wells (standard); also 0.75 mm and 1.5 mm, 10 / 15 wells
Platinum electrode	φ0.25 mm; positive (+) red, negative (−) black
Max. sample volume per well	10-well: 33 / 44 / 66 µl; 15-well: 20 / 26 / 40 µl (at 0.75 / 1.0 / 1.5 mm)
Running buffer volume	1–2 gels: 700 ml; 3–4 gels: 1000 ml
Recommended voltage	200 V constant (SDS-PAGE and most native PAGE; same setting for 2 or 4 gels)
Typical SDS-PAGE run time	Approx. 35 min at 200 V (varies with application)
Dimensions (L × W × H)	180 × 120 × 160 mm

Chapter 2 Assembly and Operation

1. Gel Plate Preparation

(1) Assembling the hand-cast glass-plate sandwich and the casting frame — please note:

(1) Stand the casting frame vertically on a level bench, with the frame door open.
(2) Select the spacer glass plate of the required gel thickness and place the plain glass plate on top of it (see Figure 3a).
(3) With the marked end of the spacer glass plate facing up, slide both plates into the casting frame so that the plain glass plate faces forward (see Figure 3b).
(4) Confirm that the two glass plates are flush and on a level plane, and that the marked orientation is correct. Incorrect plate orientation or misalignment will cause leakage.
(5) Once the plates are in position, close the casting-frame hinge to clamp the glass-plate sandwich firmly in the frame (see Figure 3c). Check that the bottom of the glass plates is flush.
(6) Keeping the casting-frame hinge facing outward, place the casting frame on the grey casting gasket of the casting stand, and press the spring lever onto the spacer glass plate (see Figure 3d).
(7) Repeat steps a–f to prepare a second gel cassette.

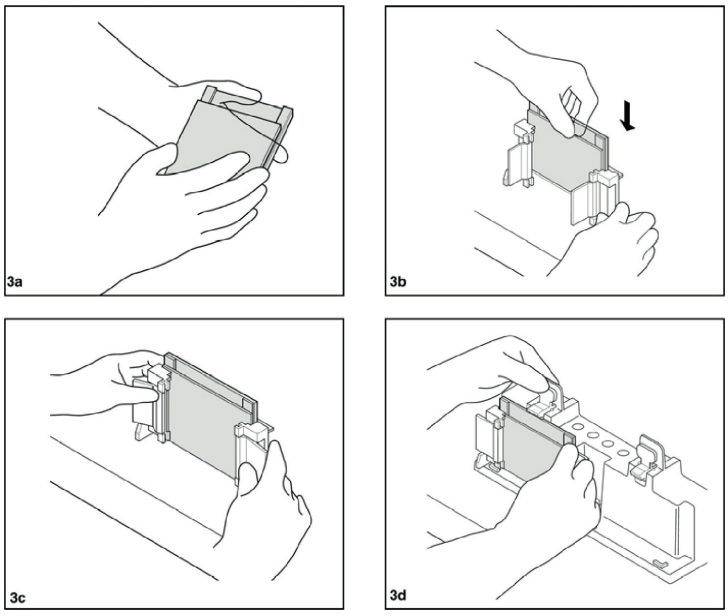

Figure 3: Assembling the casting frame and casting stand

(2) Casting the gel

Discontinuous polyacrylamide gel:

(1) Fully insert the comb into the assembled gel cassette and mark a line 1 cm below the comb teeth. This line is the height of the resolving gel.

(2) Prepare the resolving-gel monomer solution by mixing all reagents except APS and TEMED (see the gel formulation list in Chapter 4). Degas under vacuum for at least 15 minutes.

(3) Add APS and TEMED to the degassed monomer solution and, using a pipette, gently pour the solution between the glass plates up to the mark. Pour smoothly to avoid mixing air into the solution.

(4) Immediately overlay the surface with water or tert-amyl alcohol (2-methyl-2-butanol). Note: if overlaying with water, add it slowly and carefully to avoid mixing with the gel solution.

(5) Allow 45 minutes to 1 hour for the gel to polymerise. Rinse the gel surface thoroughly with double-distilled water. Do not leave alcohol on the gel for more than 1 hour, to prevent dehydration of the upper gel.

(6) (Note) The resolving gel may be stored overnight at room temperature. To prevent the resolving gel from drying out, add 5 ml of 1:4-diluted 1.5 M Tris-HCl, pH 8.8 buffer (Laemmli system). If a different buffer system is used, add 5 ml of 1× diluted resolving-gel buffer for storage.

(7) Prepare the stacking-gel monomer solution. Mix all reagents except APS and TEMED and degas under vacuum for at least 15 minutes.

(8) Before pouring the stacking gel, dry the surface of the resolving gel with filter paper.

(9) Add APS and TEMED to the degassed stacking-gel monomer solution and pour it between the glass plates until level with the plain glass plate.

(10) Insert the required comb from the top, between the side strips, making sure the tabs at both ends of the comb are guided between the strips. Insert fully until the comb spine is aligned with the plain glass plate.

(11) Allow 30–45 minutes for the stacking gel to polymerise.

(12) Gently remove the comb and rinse the gel surface thoroughly with distilled water or buffer.

(13) Rinse the used casting frame and casting stand with distilled and deionised water.

Continuous polyacrylamide gel:

(1) Prepare the gel monomer solution by mixing all reagents except APS and TEMED (see the gel formulation list in Chapter 4). Degas under vacuum for at least 15 minutes.

(2) Add APS and TEMED to the degassed monomer solution and pour it between the glass plates until level with the plain glass plate.

(3) Insert the required comb from the top, between the side strips, making sure the tabs at both ends of the comb are guided between the strips. Insert fully until the comb spine is aligned with the plain glass plate.

(4) Allow 45 minutes to 1 hour for the gel to polymerise.

(5) Gently remove the comb and rinse the gel surface thoroughly with distilled water or buffer.

(6) Rinse the used casting frame and casting stand with distilled and deionised water.

2. Electrophoresis Module Assembly and Sample Loading

Materials required: a clean, dry buffer chamber; the electrode core (the electrode-core module holds only 1 or 2 gels — for 3 or 4 gels the companion module is also required); and running buffer (700 ml for 1–2 gels; 1000 ml for 3–4 gels).

(1) Assembly

Note: When running only 2 gels, use the electrode core with the electrode plugs. When running 4 gels, use both the plug-type electrode core and the mushroom-head electrode core, with 2 gels per module.

(1) Place the gel cassette, in the open position, on a clean, level bench (see Figure 4a).

(2) Place the first gel sandwich on the gel supports — moulded into the base of the module, two on each side — with the plain glass plate facing inward. The gel plate now sits at a 30° angle to the centre. Take care when placing the first gel to keep the cassette balanced so it does not tip over. Place the second gel on the gel supports on the other side, so that the two gels are inclined toward the centre (see Figure 4b).

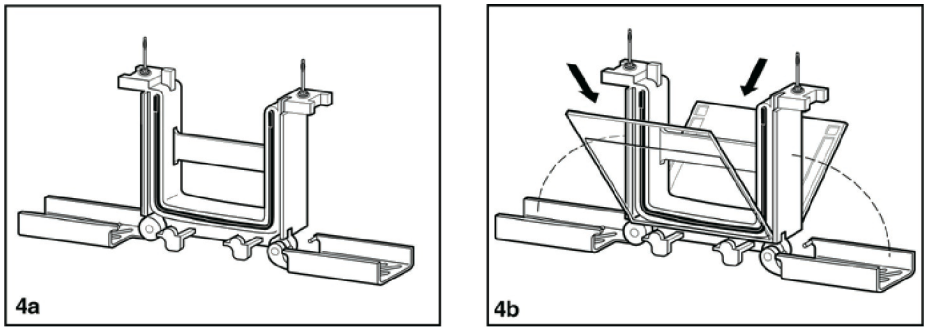

Figure 4a–4b: Loading the gel sandwiches into the electrode core

Note: The gel plates must be placed on both sides of the cassette with the plain glass plate facing inward. The cassette requires 2 gel plates to form a functional module. When running an odd number of gels (1 or 3), the single-gel replacement plate (buffer dam) must be used (see Figure 4b).

(1) With one hand, gently push the two gel plates toward the centre against the red gasket strip, making sure the plain glass plate is below the groove at the top of the red gasket.

(2) Hold the gel plates firmly with one hand and, with the other, close the red cassette clamps onto the plates until they lock into place. Alternatively, hold the whole assembly with both hands to steady the plates while closing both cassette clamps until they lock (see Figure 4c). The clamps push the plates so that the plain glass plate seats tightly against the groove of the red gasket, preventing leakage. (Confirm that the plain glass plate is below the groove at the top of the red gasket.) The sample wells can now be rinsed with buffer and loaded (see Figure 4d).

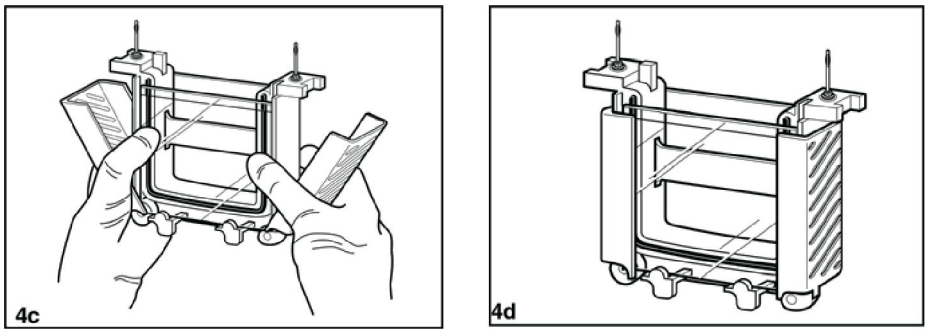

Figure 4c–4d: Clamping the cassette and the completed module

Important: Do not attempt to close the cassette clamps unless the gel plates are confirmed to be below the groove at the top of the red gasket. To prevent the plates from shifting while locking, hold them firmly and evenly against both sides of the cassette with one hand.

Note: When running 1–2 gels, do not place the mushroom-head electrode core into the cell; doing so generates extra heat and affects separation.

(2) Sample loading

(1) Add buffer to the chambers: the outer chamber up to just below the top edge of the outer glass plate, and the inner chamber until it covers the plain glass plate.

(2) Samples may be loaded either before or after the electrode core is placed in the chamber; both methods give satisfactory results.

(3) Load samples into the wells using a syringe or pipette. Load slowly so that the sample settles evenly at the bottom of the well. Do not pierce the bottom of the well with the needle or pipette tip. Buffer must be added to both the positive and negative sides; it is recommended that the buffer levels at the positive and negative electrodes be equal.

(3) Placing the electrode-core module in the buffer chamber

Note: Required buffer volume: 700 ml for 2 gels; 1000 ml for 4 gels. The buffer chamber has two positions for two modules: the plug-type electrode core at the back, the mushroom-head electrode core at the front.

(1) First, place the buffer chamber on a level bench with its front face (the face marked 2-Gels and 4-Gels) facing forward. If oriented correctly, the red mark on the chamber edge should be on the right and the black mark on the left.

(2) If running only 2 gels, use the plug-type electrode core only, placed in the rear position so that the red (+) electrode matches the red mark on the right side of the chamber.

(3) For 4 gels, in addition to the plug-type electrode core, place the mushroom-head electrode core in the front position. Confirm that the red (+) electrodes of both cores match the red

mark on the right side of the chamber. Incorrect position or orientation will prevent the upper lid from closing.

(4) Add buffer to the chamber up to the marked level.

(4) Closing the buffer chamber

Place the upper lid on the buffer chamber. Confirm that the colour-coded plugs match their sockets; the matched plugs and sockets ensure correct positioning, and the obstructions on the lid prevent incorrect positioning. The pointed projections on both sides of the buffer chamber should pass through the slots in the upper lid to ensure the lid closes correctly. Then press down on the lid with your thumbs, applying continuous force until it is firmly seated on the buffer chamber.

(5) Power conditions

(1) Insert the power leads into the power sockets of the cell, observing correct polarity (positive and negative).

(2) Power the L-VET-MINI4 to begin electrophoresis. 200 V constant voltage is the recommended condition for SDS-PAGE and most native PAGE; the same 200 V setting can be used for both 2 gels and 4 gels. Optimal voltage conditions will vary by application. Running SDS-PAGE at 200 V takes approximately 35 minutes.

(6) Gel removal

(1) When electrophoresis is complete, switch off the power and unplug the power leads.

(2) Remove the upper lid, carefully lift out the electrode core, and pour out the running buffer. To avoid spilling buffer, pour it out before opening the clamps.

(3) Open the clamps and remove the gel plates.

(4) Gently separate the two glass plates and lift the gel from the plates.

(5) With the gel face-down, immerse the gel and glass plate in transfer buffer to separate the gel from the glass plate.

(6) Rinse the electrode core, buffer chamber and other parts of the L-VET-MINI4 with distilled, deionised water.

Chapter 3 Troubleshooting

The troubleshooting guide below covers common problems encountered with polyacrylamide gel electrophoresis, their likely causes and corrective actions.

Fault symptom	Cause	Corrective action
Weak or no DNA / protein bands	Centre of the gel plate is hotter than the ends	Buffer is not mixed evenly, or the buffer in the upper chamber is too concentrated — re-prepare the buffer and ensure it is thoroughly mixed, especially when diluting 5× or 10× stock.
Weak or no bands	Power conditions too high	Reduce the set voltage from 200 V to 150 V; or add lower-chamber buffer to within 1 cm below the top edge of the plain glass plate.

Fault symptom	Cause	Corrective action
Vertical streaking of protein	Sample overloaded	Dilute the sample, selectively remove interfering proteins, or reduce the voltage by 25% to weaken the streaking.
Vertical streaking of protein	Sample precipitation	Centrifuge the sample before adding SDS sample buffer, or reduce the %T of the gel. The SDS-to-protein ratio must be sufficient to coat the protein surface with SDS — generally about 1.4:1, though some membrane proteins may require more SDS.
Lateral band spreading	Sample diffused before electrophoresis started	Minimise the time between loading and starting electrophoresis.
Lateral band spreading	Sample ionic strength lower than the gel	Use the same buffer in the sample as in the gel or stacking gel.
Distorted or skewed bands	Well polymerisation failure	Degas the stacking gel thoroughly before casting. Increase the APS and TEMED concentrations by 25%. For stacking gels and low-%T gels, the APS concentration may be kept constant while doubling the TEMED concentration.
Distorted or skewed bands	Sample contains salt	Remove salt by dialysis or with a desalting column.
Distorted or skewed bands	Uneven gel interface	Reduce the degree of polymerisation and overlay the gel surface carefully.
Lanes narrow at the bottom of the gel	Sample ionic concentration higher than the surrounding gel	Desalt the adjacent samples.
Run time markedly prolonged	Electrophoresis buffer too concentrated	Check the buffer formulation and dilute if necessary.
Run time markedly prolonged	Sample too salty	Desalt the sample.
Electrophoresis runs too fast	Electrophoresis or lower-chamber buffer too dilute	Check the buffer formulation and concentrate if necessary.
Electrophoresis runs too fast	Voltage set too high	Reduce the set voltage by 25–58%.
Two bands where a single protein is expected (SDS-PAGE)	Part of the protein may have been re-oxidised during the run, or was not fully reduced before the run	Re-prepare the sample buffer; increase the 2-mercaptoethanol concentration in the buffer; replace DTT with BME.
Fewer bands than expected, with a heavy band at the dye front	Protein migrated to the dye front	Increase the %T of the resolving gel.
Fewer bands than expected, with a heavy band at the dye front	Protein degradation	Use a protease inhibitor such as PMSF.

Fault symptom	Cause	Corrective action
Upper chamber leaks	Upper chamber overfilled	Keep the upper buffer level below the top edge of the spacer glass plate.
Upper chamber leaks	Improper assembly	Ensure the U-shaped gasket is clean and free of nicks, and that the plain glass plate is below (not above) the notch of the U-shaped gasket.
Leakage during hand-casting	Glass plate is chipped	Make sure the glass plates have no chips.
Leakage during hand-casting	Spacer plate and plain plate not level	Make sure the glass plates are correctly aligned.
Leakage during hand-casting	Casting gasket dirty, cracked or worn	Clean the casting gasket; replace it if damaged.
Poorly formed well bottoms	Incorrect catalyst composition	Prepare fresh catalyst solution, or increase the catalyst concentration in the stacking gel to 0.06% APS and 0.12% TEMED.
Pressure cam of the casting frame is hard to close or is noisy	Powder residue on the pivot of the pressure cam	Clean or wipe away powder residue before each use.

Gel parameters

A polyacrylamide gel is characterised by two parameters — the total monomer concentration (%T) and the crosslinker concentration (%C):

1) Total monomer concentration %T	= [acrylamide (g) + bis-acrylamide (g)] / total solution volume (ml) × 100%
2) Crosslinker concentration %C	= bis-acrylamide (g) / [acrylamide (g) + bis-acrylamide (g)] × 100%

Chapter 4 Warranty

The L-VET-MINI4 electrophoresis cell is provided with a one-year warranty. Any product defect caused by materials or workmanship will be repaired or replaced free of charge within the warranty period.

The warranty does not cover the following:

- Damage caused by improper operation;
- Damage caused by repair or modification performed by personnel not authorised by the company;
- General consumable parts, such as platinum wire, glass plates, casting gaskets and cell leads;
- Damage caused by the use of organic solvents.

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