

USER GUIDE

invitrogen™
by *life* technologies™

XCell4 SureLock™ Midi-Cell

For leak-free electrophoresis of 1–4 midi gels

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Revision A.0

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technologies™

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Contents

Experienced users procedure.....	iv
Kit contents.....	vi
Introduction	1
Product description.....	1
Description of parts.....	2
Experimental overview.....	4
Methods	5
General guidelines for preparing samples and buffers.....	5
Assemble the XCell4 SureLock™ Midi-Cell.....	7
Perform electrophoresis.....	13
Disassemble the XCell4 SureLock™ Midi-Cell.....	14
Troubleshooting.....	16
Appendix.....	17
Safety information.....	17
Sicherheits-Anweisungen.....	19
Instructions de sécurité.....	20
Product specifications.....	21
Accessory products.....	22
Technical support.....	23

Experienced users procedure

Introduction

A brief experienced users procedure for performing electrophoresis with the XCell4 SureLock™ Midi-Cell is described below. If you are a first time user of the XCell4 SureLock™ Midi-Cell, refer to the detailed protocols in this manual.

Action	Procedure												
Prepare sample and buffer	<p>Prepare protein sample at the appropriate concentration using the NuPAGE® LDS Sample Buffer or Tris-Glycine SDS Sample Buffer for denaturing SDS-PAGE and using the Tris-Glycine Native Sample Buffer for native PAGE (for use with NuPAGE® Midi Tris-Acetate Midi and Tris-Glycine Midi Gels only).</p> <p>Prepare the following 1X Running Buffer (see page 5 for the amount of buffer needed based on the number of gels used).</p> <table border="1" data-bbox="440 667 1443 1129"> <tbody> <tr> <td data-bbox="440 667 873 747">NuPAGE® Bis-Tris Midi Gels</td> <td data-bbox="873 667 1443 747">1X NuPAGE® MES SDS Running Buffer to resolve small proteins</td> </tr> <tr> <td data-bbox="440 747 873 827"></td> <td data-bbox="873 747 1443 827">1X NuPAGE® MOPS SDS Running Buffer to resolve mid-size proteins</td> </tr> <tr> <td data-bbox="440 827 873 974">NuPAGE® Tris-Acetate Midi Gels</td> <td data-bbox="873 827 1443 974">1X NuPAGE® Tris-Acetate SDS Running Buffer to resolve large proteins</td> </tr> <tr> <td data-bbox="440 974 873 1054"></td> <td data-bbox="873 974 1443 1054">1X Tris-Glycine Native Running Buffer to perform native gel electrophoresis</td> </tr> <tr> <td data-bbox="440 1054 873 1129">Tris-Glycine Midi Gels</td> <td data-bbox="873 1054 1443 1129">1X Tris-Glycine SDS Running Buffer for denaturing SDS-PAGE</td> </tr> <tr> <td data-bbox="440 1129 873 1209"></td> <td data-bbox="873 1129 1443 1209">1X Tris-Glycine Native Running Buffer for native gel electrophoresis</td> </tr> </tbody> </table>	NuPAGE® Bis-Tris Midi Gels	1X NuPAGE® MES SDS Running Buffer to resolve small proteins		1X NuPAGE® MOPS SDS Running Buffer to resolve mid-size proteins	NuPAGE® Tris-Acetate Midi Gels	1X NuPAGE® Tris-Acetate SDS Running Buffer to resolve large proteins		1X Tris-Glycine Native Running Buffer to perform native gel electrophoresis	Tris-Glycine Midi Gels	1X Tris-Glycine SDS Running Buffer for denaturing SDS-PAGE		1X Tris-Glycine Native Running Buffer for native gel electrophoresis
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Tris-Glycine Midi Gels	1X Tris-Glycine SDS Running Buffer for denaturing SDS-PAGE												
	1X Tris-Glycine Native Running Buffer for native gel electrophoresis												
Prepare gel cassette	<ol style="list-style-type: none"> 1. Remove the gel cassette from the pouch and rinse with deionized water. 2. Peel off the tape covering the slot on the back of the gel cassette and pull the comb out of the cassette. Rinse the wells with 1X running buffer and fill the sample wells with running buffer. 												
Assemble with 4 gels	<p>Instructions for assembling four gels are described below. For assembling less than four gels, see page 9.</p> <ol style="list-style-type: none"> 1. Insert the XCell4 SureLock™ Assembly in its unlocked position into the center of the Midi-Cell base. The XCell4 SureLock™ Assembly slides down over the protrusion in the Midi-Cell base. 2. Place one cassette on each side of both Buffer Cores. 3. While holding the assembly together with your hands, insert the Buffer Cores with the gel cassettes into the Lower Buffer Chamber such that the negative electrode fits into the opening in the gold plate on the Lower Buffer Chamber. Always hold the cassette assembly by its edges. 4. Lock the XCell4 SureLock™ Assembly by moving the tension lever to the locked position (indicated on the XCell4 SureLock™ Assembly). This will squeeze the gels and Buffer Cores together, creating leak free seals. 												

Continued on next page

Experienced users procedure, continued

Action	Procedure																					
Add buffer and load samples	<ol style="list-style-type: none"> 1. Fill each of the Upper Buffer Chambers with 175 mL of the appropriate 1X running buffer. For running NuPAGE® Midi Gels under reducing conditions, use 1X Running Buffer with 435 µL NuPAGE® Antioxidant in each of the Upper Buffer Chambers. Ensure that the Upper Buffer Chambers are not leaking. 2. Load the appropriate volume of the protein sample and molecular weight markers in the wells. 3. Add 700 mL 1X running buffer (for 4 gels) to the Lower Buffer Chamber (anode) by pouring into the center of the Midi-Cell (over the XCell4 SureLock™ Assembly). Fill to the fill line marked on the Midi-Cell. 4. Place the lid on the assembled XCell4 SureLock™ Midi-Cell. The lid firmly seats if the (-) and (+) electrodes are properly aligned. 5. With the power off, connect the electrode cords to power supply. Turn on the power and perform electrophoresis as follows. <table border="1" data-bbox="451 779 1360 1270"> <thead> <tr> <th data-bbox="451 779 954 821">Gel type</th> <th data-bbox="959 779 1192 821">Voltage</th> <th data-bbox="1196 779 1360 821">Run time</th> </tr> </thead> <tbody> <tr> <td data-bbox="451 827 954 898">NuPAGE® Bis-Tris SDS-PAGE with MES Running Buffer</td> <td data-bbox="959 827 1192 898">1. 200 V Constant</td> <td data-bbox="1196 827 1360 898">40 min</td> </tr> <tr> <td data-bbox="451 905 954 976">NuPAGE® Bis-Tris SDS-PAGE with MOPS Running Buffer</td> <td data-bbox="959 905 1192 976">200 V Constant</td> <td data-bbox="1196 905 1360 976">55 min</td> </tr> <tr> <td data-bbox="451 982 954 1054">NuPAGE® Tris-Acetate SDS-PAGE (denaturing, reducing)</td> <td data-bbox="959 982 1192 1054">150 V Constant</td> <td data-bbox="1196 982 1360 1054">70 min</td> </tr> <tr> <td data-bbox="451 1060 954 1131">NuPAGE® Tris-Acetate SDS-PAGE (native)</td> <td data-bbox="959 1060 1192 1131">150 V Constant</td> <td data-bbox="1196 1060 1360 1131">2 hr–3 hr</td> </tr> <tr> <td data-bbox="451 1138 954 1209">Tris-Glycine with SDS Running Buffer (denaturing, reducing)</td> <td data-bbox="959 1138 1192 1209">125 V Constant</td> <td data-bbox="1196 1138 1360 1209">105 min</td> </tr> <tr> <td data-bbox="451 1215 954 1270">Tris-Glycine with Tris-Glycine Native Running Buffer (native electrophoresis)</td> <td data-bbox="959 1215 1192 1270">125 V Constant</td> <td data-bbox="1196 1215 1360 1270">1 hr–12 hr</td> </tr> </tbody> </table> 	Gel type	Voltage	Run time	NuPAGE® Bis-Tris SDS-PAGE with MES Running Buffer	1. 200 V Constant	40 min	NuPAGE® Bis-Tris SDS-PAGE with MOPS Running Buffer	200 V Constant	55 min	NuPAGE® Tris-Acetate SDS-PAGE (denaturing, reducing)	150 V Constant	70 min	NuPAGE® Tris-Acetate SDS-PAGE (native)	150 V Constant	2 hr–3 hr	Tris-Glycine with SDS Running Buffer (denaturing, reducing)	125 V Constant	105 min	Tris-Glycine with Tris-Glycine Native Running Buffer (native electrophoresis)	125 V Constant	1 hr–12 hr
Gel type	Voltage	Run time																				
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NuPAGE® Tris-Acetate SDS-PAGE (denaturing, reducing)	150 V Constant	70 min																				
NuPAGE® Tris-Acetate SDS-PAGE (native)	150 V Constant	2 hr–3 hr																				
Tris-Glycine with SDS Running Buffer (denaturing, reducing)	125 V Constant	105 min																				
Tris-Glycine with Tris-Glycine Native Running Buffer (native electrophoresis)	125 V Constant	1 hr–12 hr																				
Disassemble	<ol style="list-style-type: none"> 1. Remove the lid. Discard the buffer. 2. Unlock the XCell SureLock® Assembly by moving the tension lever to the unlocked position (indicated on the XCell SureLock® Assembly). 3. Remove the Buffer Cores with gel cassettes from the Lower Buffer Chamber while holding the cassettes against the cores. 4. Remove the gel cassettes from Buffer Cores and carefully insert the gel knife (included with the Midi-Cell) beveled edge into the narrow gap between the two plates of the cassette. Caution: Use caution while inserting the gel knife between the two plates to avoid excessive pressure towards the gel. 5. Push up and down gently on the knife's handle to separate the plates. Repeat on each side of the cassette until the plates are completely separated. 6. Upon opening the cassette, remove and discard the plate without the gel, allowing the gel to remain on the other plate. 7. If blotting, proceed to the western transfer protocol (refer to XCell II™ Blot Module manual for more details). For staining, remove the bottom foot of the gel with a Gel Knife, if desired. Hold the cassette plate and gel over a container with the gel facing downward. Use the gel knife to carefully loosen one lower corner of the gel and allow the gel to peel away from the plate. 																					

Kit contents

Contents

The components included with the XCell4 SureLock™ Midi-Cell are listed below. For more details on each component, see page 2.

Components	Quantity
XCell4™ Lower Buffer Chamber	1 each
XCell4™ Lid with Cables	1 each
XCell4™ Buffer Cores	2 each
XCell4™ Buffer Dam	1 each
XCell4™ Displacement Dam	1 each
XCell4 SureLock™ Assembly	1 each
Gel Knife	1 each

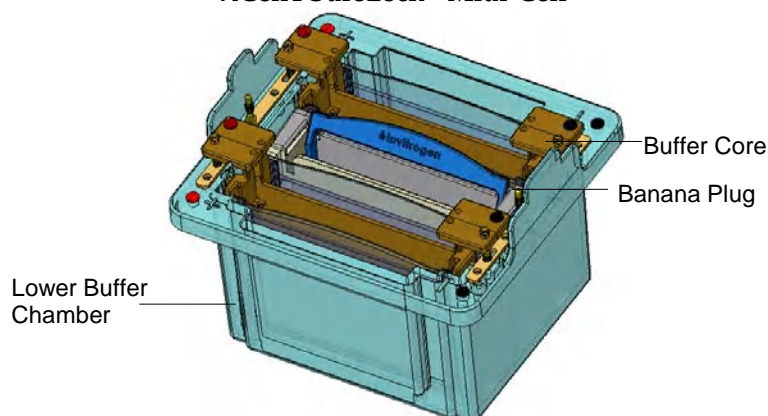
Introduction

Product description

XCell4 SureLock™ Midi-Cell

The XCell4 SureLock™ Midi-Cell's unique design allows you to quickly and easily perform simultaneous vertical electrophoresis of 1–4 midi-gels without leaking, resulting in consistent performance. The XCell4 SureLock™ Midi-Cell is built with tough plastic material to boost durability and features a specially designed lid to enhance user safety.

XCell4 SureLock™ Midi-Cell



System overview

The XCell4 SureLock™ Midi-Cell consists of a Lower Buffer Chamber, two Buffer Cores, Buffer Dam, Displacement Dam, and a lid with cables (see page 2 for detailed description of parts). Electrophoresis of proteins on midi-gels in the XCell4 SureLock™ Midi-Cell is performed after assembling each Buffer Core with 2 midi-gels or a Buffer Core with a midi-gel and a Buffer Dam. The Buffer Core/gel assembly is inserted into the Lower Buffer Chamber and is sealed using a positive locking mechanism that seals the Buffer Core/gel assembly firmly into position and ensures leak-free electrophoresis. The Buffer Core/gel assembly creates separate upper buffer chambers for each gel and a common lower buffer chamber shared by all gels. The lid is placed onto the Midi-Cell and fits on the Midi-Cell in only one orientation to prevent incorrect connection to the electrodes.

The unique design of the XCell4 SureLock™ Midi-Cell evenly dissipates heat resulting in consistent performance of all gels without the need for any external cooling device. The Buffer Core electrical poles are color-coded for easy, intuitive assembly.

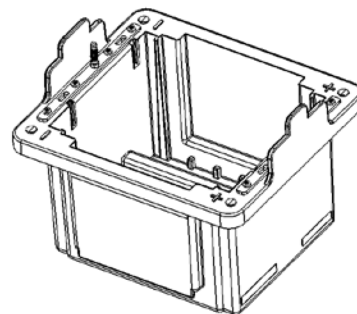
Features

- User-friendly design without any clamps or grease for leak-free electrophoresis
 - Flexibility to perform electrophoresis of 1–4 midi-gels
 - Unique, heat-dissipating design of the XCell4 SureLock™ Midi-Cell produces consistent results without the need for any cooling device
 - Built-in safety features to enhance user safety
-

Description of parts

XCell4™ Lower Buffer Chamber

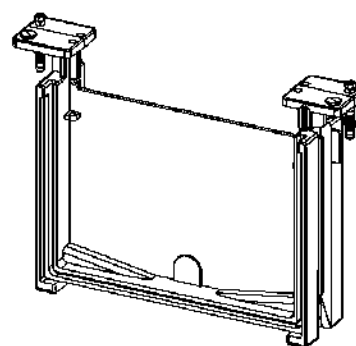
The XCell4™ Lower Buffer Chamber, when assembled with the XCell4 SureLock™ Assembly and Buffer Cores, creates two individual chambers for electrophoresis. The Lower Buffer Chamber volume is 700 mL and is used as a heat sink and ion reservoir during electrophoresis. The Lower Buffer Chamber when combined with the XCell4™ Lid produces an enclosed unit for electrophoresis.



XCell4™ Buffer Core

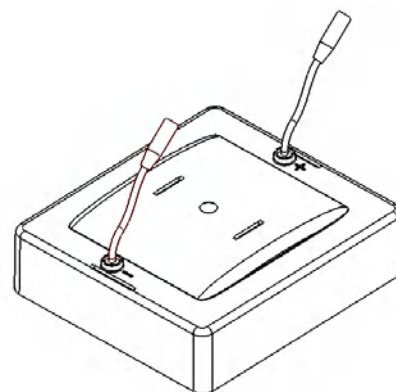
The unique design of the XCell4™ Buffer Core with the XCell4 SureLock™ Assembly provides a consistent seal without the use of any clamps or grease. The Buffer Core is specifically designed to evenly dissipate heat resulting in consistent performance across all gels. The Buffer Core contains the anode and cathode electrode assembly and has color-coded electrical poles for easy, intuitive assembly.

The XCell4 SureLock™ Midi-Cell includes 2 Buffer Cores.



XCell4™ Lid with cables

The XCell4™ Lid will fit onto the Midi-Cell in only one orientation to prevent incorrect connection to the electrodes.



Gel Knife

The Gel Knife is used to open the gel cassette to remove the gel.

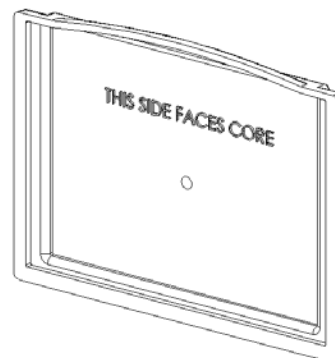


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Description of parts, continued

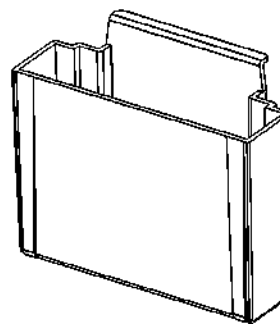
XCell4™ Buffer Dam

Use the XCell4™ Buffer Dam when you wish to run less than 4 gels in the XCell4 SureLock™ Midi-Cell. The Buffer Dam is placed in the Buffer Core instead of a second gel. Instructions for using the Buffer Dam to run 1 or 3 Midi Gels are provided on page 9.



XCell4™ Displacement Dam

Use the XCell4™ Displacement Dam when you wish to run less than 3 gels in the XCell4 SureLock™ Midi-Cell. The Displacement Dam is placed in the Lower Buffer Chamber instead of a second Buffer Core. Instructions for using the Displacement Dam to run 1 or 2 midi-gels are provided on page 9. **Do not add any buffer or water to the Displacement Dam.**



XCell SureLock® Assembly

The positive locking action of the XCell SureLock® Assembly ensures trouble-free, leak-free electrophoresis of protein gels. Move the lever on the XCell SureLock® Assembly to a locked position to generate an even horizontal force which seals the gel cassette/Buffer Core assembly firmly into position in the Lower Buffer Chamber. The locked and unlocked positions are marked (shown with an arrow in figure A) on the XCell SureLock® Assembly for easy, intuitive assembly. Figure B shows the markings in detail.

Note: Do not use the Gel Tension Wedge supplied with the XCell SureLock® Mini-Cell with the Midi-Cell as the wedge is specifically designed for the Mini-Cell and will not fit correctly in the Midi-Cell.

Figure A

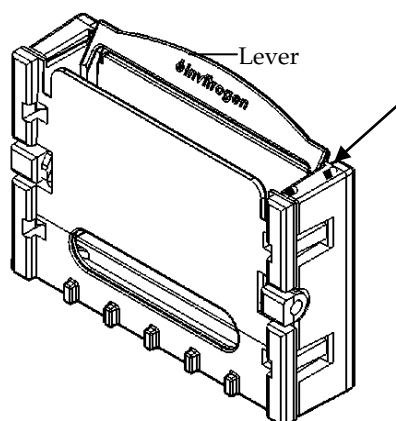
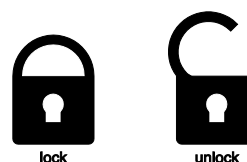


Figure B

The locked and unlocked positions marked on the assembly are shown in detail below.



Experimental overview

Experimental outline

The table below outlines the experimental steps necessary to perform electrophoresis using the XCell4 SureLock™ Midi-Cell. For more details on each step, see indicated pages.

Step	Action	Page
1	Prepare protein samples.	5
2	Prepare running buffer.	5
3	Assemble the XCell4 SureLock™ Midi-Cell with the desired number of gel cassettes.	7
4	Load the 1X Running Buffer into the upper buffer chamber.	10
5	Load protein samples into the wells of the gel. Load running buffer into the Lower Buffer Chamber.	11
6	Perform electrophoresis using the recommended run conditions.	13
7	Disassemble the XCell4 SureLock™ Midi-Cell.	14

Materials needed

Ordering information is on page 22.

- Power supply (see the following section)
- Novex® Midi Gels
- Gel loading tips
- Protein samples
- Protein Standard
- Sample buffer (see page 5)
- Running buffer (see page 5)

Power supply requirements

The XCell4 SureLock™ Midi-Cell is used with an external DC power supply designed for electrophoresis. This power supply must:

- Be isolated from the ground so that the DC output is floating and must be equipped with No Load or Open Circuit protection
- Be able to operate at 200 V

The ZOOM® Dual Power Supply and PowerEase® 90W Power Supply and PowerEase® 300W Power Supply are compatible for use with Novex® Midi Gels. See page 22 for ordering information.

Note: The XCell4 SureLock™ Midi-Cell has covered power leads to be in compliance with the latest IEC (International Electrotechnical Commission) and EN (European) standards, and reduce the risk of electrical shock due to exposure to bare metal. To allow connection of the XCell4 SureLock™ Midi-Cell covered power leads with certain power supplies, use Novex® Power Supply Adapters available separately (page 22).

Methods

General guidelines for preparing samples and buffers

Introduction

General guidelines for preparing samples and buffers for Novex® Midi precast gels are discussed below.

Detailed instructions for preparing the sample buffer and running buffer for each gel type is provided in the instruction manual included with each gel type. This manual is available for downloading from our web site at www.lifetechnologies.com or by contacting Technical Support (page 23).

Recommended buffers

The recommended running buffer and sample buffer for each Novex® Midi Gel is listed below. You will prepare your sample in the appropriate sample buffer such that the final concentration of the sample buffer is 1X. You need to dilute the running buffer to 1X final concentration before use. See page 22 for ordering information on pre-mixed buffers.

Gel type	Running buffer	Sample buffer
NuPAGE® Novex® Bis-Tris Midi Gels	NuPAGE® MES SDS Running Buffer (20X) to resolve small proteins	NuPAGE® LDS Sample Buffer (4X)
	NuPAGE® MOPS SDS Running Buffer (20X) to resolve mid-size proteins	NuPAGE® LDS Sample Buffer (4X)
NuPAGE® Novex® Tris-Acetate Midi Gels	NuPAGE® Tris-Acetate SDS Running Buffer (20X) to resolve large proteins	NuPAGE® LDS Sample Buffer (4X)
	Tris-Glycine Native Running Buffer (10X) to perform native gel electrophoresis	Tris-Glycine Native Sample Buffer (2X)
Novex® Tris-Glycine Midi Gels	Tris-Glycine SDS Running Buffer (10X) to perform denaturing gel electrophoresis	Tris-Glycine SDS Sample Buffer (2X)
	Tris-Glycine Native Running Buffer (10X) to perform native gel electrophoresis	Tris-Glycine Native Sample Buffer (2X)

Amount of buffer

The amount of 1X Running Buffer required depends on the number of gels used for electrophoresis as described below:

Number of gels	Amount of buffer
4	1400 mL
3	1250 mL
2	950 mL
1	750 mL

Continued on next page

General guidelines for preparing samples and buffers, continued

Reducing agent

The NuPAGE® Sample Reducing Agent contains 500 mM dithiothreitol (DTT) at a 10X concentration in a ready-to-use, stabilized liquid form (page 22 for ordering information) and is used to reduce samples for electrophoresis.

β -mercaptoethanol can be used with the Novex® Midi gels at a final concentration of 2.5% (v/v). Choice of the reducing agent is a matter of preference and either DTT or β -mercaptoethanol can be used. **We recommend adding the reducing agent to the sample within an hour of loading the gel.**

Avoid storing reduced samples for long periods of time even if they are frozen. This will result in the reoxidation of samples during storage and produce inconsistent results.

Important

Do not use the NuPAGE® Antioxidant (see below) as a sample reducing agent. The antioxidant is not efficient in reducing the disulfide bonds. This will result in partially reduced bands with substantial background smearing in the lane.

NuPAGE® Antioxidant

The NuPAGE® Antioxidant (a proprietary reagent) is added to the running buffer in the upper (cathode) buffer chamber only when performing electrophoresis of NuPAGE® Novex® Midi Gels under reducing conditions. The NuPAGE® Antioxidant migrates with the proteins during electrophoresis preventing the proteins from reoxidizing and maintaining the proteins in a reduced state. The NuPAGE® Antioxidant also protects sensitive amino acids such as methionine and tryptophan from oxidizing.

The NuPAGE® Antioxidant is **not** compatible with gel systems other than the NuPAGE® system as the antioxidant is not efficient at higher pH of other gel systems including Novex® Tris-Glycine Midi Gels.

If you are performing electrophoresis of NuPAGE® Novex Midi Gels under reducing conditions using the XCell4 *SureLock*™ Midi-Cell, use 175 mL 1X Running Buffer with 435 μ L NuPAGE® Antioxidant in each of the Upper Buffer Chambers.

Assemble the XCell4 SureLock™ Midi-Cell

Materials needed

- Appropriate precast gels (page 21)
 - Running buffer (1X)
 - Protein sample and protein standard
 - Gel loading tips
-

First time use

The Buffer Cores and lid of the XCell4 SureLock™ Midi-Cell are supplied assembled.

If you are using the XCell4 SureLock™ Midi-Cell for the first time, you need to disassemble the XCell4 SureLock™ Midi-Cell and wash various parts before use as described below:

1. Remove the lid.
 2. Remove the Buffer Dam, Displacement Dam, and 2 Buffer Cores from the Lower Buffer Chamber.
 3. Wash various parts of the XCell4 SureLock™ Midi-Cell with a mild detergent and rinse with deionized water before use. Do not damage the wires on the core during the washing.
-



To avoid contamination from possible residual acrylamide, wear gloves and laboratory coat when loading and unloading gels into the XCell4 SureLock™ Midi-Cell, and when staining, destaining, or drying gels.

Prepare gel cassettes

1. Cut open the gel cassette pouch and drain away the gel packaging buffer.
2. Remove the gel cassette from the pouch and rinse with deionized water.
3. Peel off the tape covering the slot on the back of the gel cassette.
4. In one fluid motion, pull the comb out of the cassette.
5. Use a pipette to gently wash the cassette wells with 1X running buffer. Invert the gel and shake to remove buffer. Repeat twice. Fill the sample wells with running buffer. Be sure to displace all air bubbles from the cassette wells, as they will affect sample running.

Note: Always handle the cassette by its edges only.

Note

To obtain the best results, use gels of the same type and same percentage of acrylamide in the XCell4 SureLock™ Midi-Cell. **Avoid mixing different gel types and percentage of gels.**

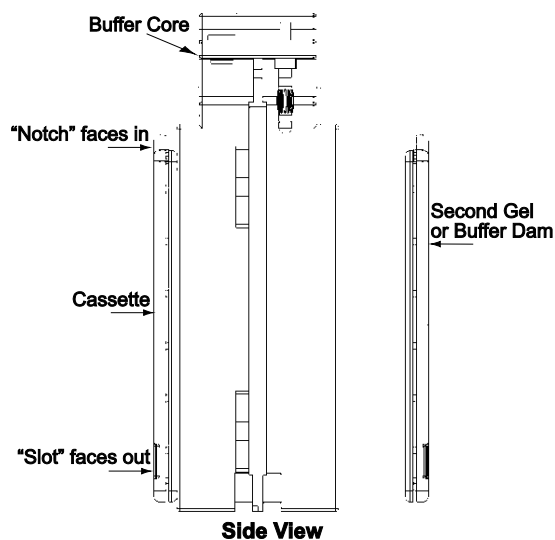
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Assemble the XCell4 SureLock™ Midi-Cell, continued

Assemble with 4 gels

Instructions are provided below to assemble the XCell4 SureLock™ Midi-Cell with 4 precast midi-gels. See page 9 to assemble with less than 4 gels.

1. Insert the XCell4 SureLock™ Assembly in its unlocked position into the center of the Midi-Cell base. The XCell4 SureLock™ Assembly slides down over the protrusion in the Midi-Cell base.
2. Place one cassette on each side of the Buffer Core for each of the two cores. For each cassette, the shorter “well” side of the cassette faces **in** towards the buffer core. The slot on the back of the cassette must face out towards the lower buffer chamber (see the following figure).



3. While holding the assembly together with your hands (Figure A), insert the Buffer Cores with the gel cassettes into the Lower Buffer Chamber such that the negative electrode fits into the opening in the gold plate on the Lower Buffer Chamber (Figure B). Always hold the cassette assembly by its edges as shown in the following figure.



Note: If you are having difficulty inserting the assembly into the lower buffer chamber, make sure the cathode (black polarity indicator) of the Buffer Core is aligned with the cathode (black polarity indicator) of the Lower Buffer Chamber.

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Assemble the XCell4 SureLock™ Midi-Cell, continued

Assemble with 4 gels, continued

Procedure continued from previous page.

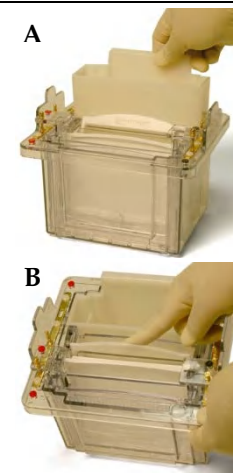
4. The Upper Buffer Chamber (cathode) is the void formed between a gel and the Buffer Core at the center of each core.
5. Lock the XCell4 SureLock™ Assembly by moving the tension lever to the locked position (indicated on the XCell4 SureLock™ Assembly). This will squeeze the gels and Buffer Cores together, creating leak free seals.
6. Proceed to loading samples and buffers, pages 10–12.

Assemble with less than 4 gels

Instructions are provided below to assemble the XCell4 SureLock™ Midi-Cell with less than 4 gels using the Buffer Dam and Displacement Dam included with the Midi-Cell.

Note: Do not use the XCell SureLock® Buffer Dam with the XCell4 SureLock™ Midi-Cell.

Gel Number	2. Instructions
Assemble with 3 gels	<ol style="list-style-type: none"> 1. Insert the XCell4 SureLock™ Assembly in its unlocked position into the Midi-Cell. The XCell4 SureLock™ Assembly should rest on the bottom of the Lower Buffer Chamber. 2. Assemble 1 Buffer Core with 2 gel cassettes and insert the Buffer Core/gel cassette assembly into chamber 1 of the Lower Buffer Chamber as described on page 8. 3. Assemble the second Buffer Core with a gel cassette and Buffer Dam (see adjacent figure) and insert into chamber 2 of the Lower Buffer Chamber. 4. Lock the XCell4 SureLock™ Assembly by moving the tension lever to the locked position (indicated on the XCell4 SureLock™ Assembly) to create a leak free seal.
Assemble with 2 gels	<ol style="list-style-type: none"> 1. Insert the XCell4 SureLock™ Assembly in its unlocked position into the Midi-Cell between the two Buffer Cores. The XCell4 SureLock™ Assembly should rest on the bottom of the Lower Buffer Chamber. 2. Assemble 1 Buffer Core with 2 gel cassettes and insert the Buffer Core/gel cassette assembly into chamber 1 of the Lower Buffer Chamber as described on page 8. 3. Insert the Displacement Dam into chamber 2 of the Lower Buffer Chamber (figure A). 4. Lock the XCell4 SureLock™ Assembly by moving the tension lever to the locked position (indicated on the XCell4 SureLock™ Assembly) to create a leak free seal (figure B).



Continued on next page

Assemble the XCell4 SureLock™ Midi-Cell, continued

Assemble with less than 4 gels, continued

Gel number	Instructions
Assemble with 1 gel	<ol style="list-style-type: none"><li data-bbox="391 432 1406 520">1. Insert the XCell4 SureLock™ Assembly in its unlocked position into the Midi-Cell between the two Buffer Cores. The XCell4 SureLock™ Assembly should rest on the bottom of the Lower Buffer Chamber.<li data-bbox="391 531 1360 594">2. Assemble 1 Buffer Core with 1 gel cassette and 1 Buffer Dam and insert the assembly into chamber 1 of the Lower Buffer Chamber as described on page 8.<li data-bbox="391 604 1349 667">3. Insert the Displacement Dam into chamber 2 of the Lower Buffer Chamber as described on the previous page.<li data-bbox="391 678 1382 732">4. Lock the XCell4 SureLock™ Assembly by moving the tension lever to the locked position (indicated on the XCell4 SureLock™ Assembly) to create a leak free seal.

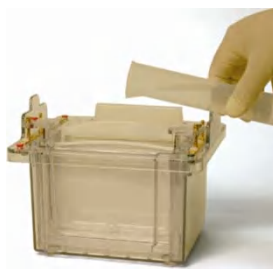
Add buffer into the Upper Buffer Chamber

After assembling the XCell4 SureLock™ Midi-Cell, load buffer in the Upper Buffer Chamber as described below. The Upper Buffer Chamber (cathode) is the void formed between a gel and Buffer Core at the center of each core.

1. Fill **each** of the Upper Buffer Chambers assembled with gels with 175 mL of the appropriate 1X running buffer (page 5). Use enough running buffer to completely cover the sample wells. If using only the Displacement Dam, there is no need to add any buffer to the Dam.
2. If you are running NuPAGE® Novex Midi Gels under reducing conditions, use 1X Running Buffer with 435 µL NuPAGE® Antioxidant in each of the Upper Buffer Chambers.

Tip: Slowly load the buffer into the Upper Buffer Chamber to avoid a lot of bubble formation. Bubbles make sample loading more difficult.

3. Ensure that the Upper Buffer Chambers are not leaking. If the level of running buffer drops, the electrophoresis core and cassettes are not properly seated. Disassemble the Buffer Core/cassette assembly and repeat the assembly.



Continued on next page

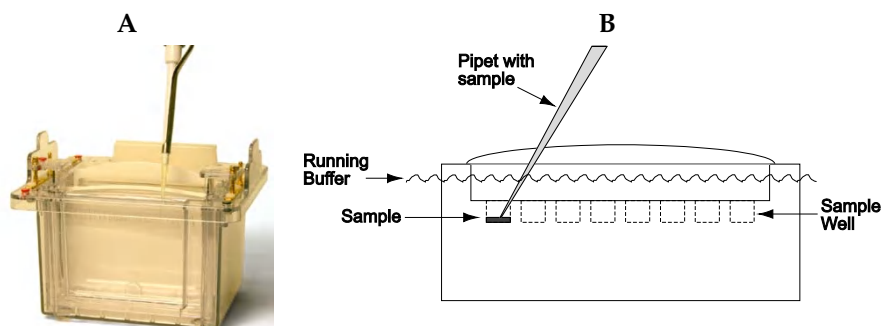
Assemble the XCell4 SureLock™ Midi-Cell, continued

Load samples

The recommended sample volume for each Novex® Midi gel type is listed on page 21.

1. Prepare your samples using the appropriate sample buffer at the desired protein concentration.
2. Use a pipette equipped with a sample loading tip (page 22) to underlay the samples into the gel wells. Lower the tip to the bottom of the sample well and slowly pipet sample into well without contaminating another well with the sample (see figures A and B below). The sample wells are marked on the cassette allowing you to easily identify the sample wells and load the samples properly into each well.

Note: To obtain the best results and promote a uniform running of the stacking front, load sample buffer in all the wells, whether or not they contain samples.



Important

Do not add any buffer or water to the Displacement Dam.

However, if you have accidentally added any water or buffer to the Displacement Dam, it does not affect the electrophoresis run.


Continued on next page

Assemble the XCell4 SureLock™ Midi-Cell, continued


Add buffer into the Lower Buffer Chamber

1. Add the appropriate amount of 1X running buffer as described below to the Lower Buffer Chamber (anode) by pouring into the center of the Midi-Cell (over the XCell4 SureLock™ Assembly). Fill to the fill level marked on the outside of the Midi-Cell.

Number of gels	1X Running Buffer
4	700 mL
3	680 mL
2	560 mL
1	540 mL

-  Do not handle the lid if the cables are plugged into the power supply.
2. Place the lid on the assembled XCell4 SureLock™ Midi-Cell. The lid will firmly seat if the (-) and (+) electrodes are properly aligned. If the lid is not properly seated, no power will go through the Midi-Cell.



3. With the power **off**, connect the electrode cords to power supply [red to (+) jack, black to (-) jack].
 -  Make sure the power is off before connecting the XCell4 SureLock™ Midi-Cell to the power supply.
 4. Turn on the power. See next page for electrophoresis conditions.
-

Perform electrophoresis

Electrophoresis conditions

Once you have assembled the XCell4 SureLock™ Midi-Cell and loaded your samples, you are ready to perform electrophoresis.

You will need a power supply designed for electrophoresis (page 4).

The electrophoresis conditions for different Novex® Midi Gels using the XCell4 SureLock™ Midi-Cell are listed below.

Gel type	Voltage	Expected current*	Run time
NuPAGE® Bis-Tris SDS-PAGE (denaturing, reducing) with MES Running Buffer	200 V Constant	Start: 160–200 mA End: 120–170 mA	40 min
NuPAGE® Bis-Tris SDS-PAGE (denaturing, reducing) with MOPS Running Buffer	200 V Constant	Start: 160–200 mA End: 120–170 mA	55 min
NuPAGE® Tris-Acetate SDS-PAGE (denaturing, reducing)	150 V Constant	Start: 70–90 mA End: 50–60 mA	70 min
NuPAGE® Tris-Acetate SDS-PAGE (native)	150 V Constant	Start: 40–45 mA End: 15–20 mA	2 hr–3 hr
Tris-Glycine with Tris-Glycine SDS Running Buffer	125 V Constant	Start: 40–50 mA End: 20–25 mA	105 min
Tris-Glycine with Tris-Glycine Native Running Buffer (native electrophoresis)	125 V Constant	Start: 35–40 mA End: 15–20 mA	1 hr–12 hr

*Current readings are per gel

At the end of the run, turn off the power, and disconnect cables from the power supply. Proceed immediately to **Disassemble the XCell4 SureLock™ Midi-Cell**, page 14.

Disassemble the XCell4 SureLock™ Midi-Cell

Procedure

 Be sure the cables are disconnected from the power supply.

1. Remove the lid. Discard the buffer.
2. Unlock the XCell4 SureLock™ Assembly by moving the tension lever to the unlocked position (indicated on the XCell4 SureLock™ Assembly).

Note: Moving the tension lever to the unlocked position before discarding the buffer from the Midi-Cell may cause some splashing of the buffer. Prior to unlocking the XCell4 SureLock™ Assembly, be sure to **discard** the buffer.

3. Remove the Buffer Cores with the gel cassettes from the Lower Buffer Chamber while holding the cassettes against the cores as shown in the following figure.



4. Remove the gel cassettes from the Buffer Cores and lay the gel cassettes (well side up) on a flat surface, such as the benchtop. Allow one edge to hang ~1 cm over the side of the benchtop.
5. Carefully insert the Gel Knife's (included with the Midi-Cell) beveled edge into the narrow gap between the two plates of the cassette.

Caution: Use caution while inserting the Gel Knife between the two plates to avoid excessive pressure towards the gel.

6. Push up and down gently on the knife's handle to separate the plates. You will hear a cracking sound indicating that you have broken the bonds which hold the plates together. Repeat on each side of the cassette until the plates are completely separated.
7. Upon opening the cassette, the gel may adhere to either side. Remove and discard the plate without the gel, allowing the gel to remain on the other plate.

Note: The Cassette Post (small plastic piece near the top of the cassette) may remain on either plate of the cassette after opening the two plates of the cassette. The Cassette Post is designed to maintain proper electrophoresis conditions that result in optimal separation and does not interfere with the sample loading or electrophoresis run.

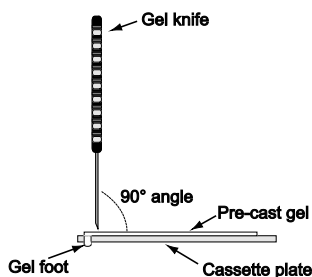
8. If blotting, proceed to the western transfer protocol (refer to the Novex® Midi Gel System manual for more details).

Continued on next page

Disassemble the XCell4 SureLock™ Midi-Cell, continued

Procedure, continued

9. If staining, remove the gel from the cassette plate by one of the two methods:
 - If the gel remains on the shorter (notched) plate, use the sharp edge of the Gel Knife to remove the bottom foot of the gel, if desired. Hold the Gel Knife at a 90° angle to the gel and the slotted cassette plate. Push straight down on the knife to cut the gel. Repeat the motion across the gel to cut the entire foot. Hold the cassette plate and gel over a container with the gel facing downward. Use the knife to carefully loosen one lower corner of the gel and allow the gel to peel away from the plate.



- If the gel remains on the longer (slotted) plate, hold the cassette plate and gel over a container with the gel facing downward. Gently push the gel knife through the slot in the cassette, until the gel peels away from the plate. Cut the foot off the gel after fixing and staining, but before drying.
10. Immediately stain the gel as desired.

Cleaning and maintenance

Wash the XCell4 SureLock™ Midi-Cell with a mild detergent and rinse with deionized water after each use.

If the wires on the Buffer Cores are damaged, you can replace the wire using the spare wire available separately (page 21).

For more information, visit www.lifetechnologies.com or contact Technical Support (page 23). **Do not** perform any repairs or service on the XCell4 SureLock™ Midi-Cell to avoid damaging the Midi-Cell.

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments using the XCell4 SureLock™ Midi-Cell.

Observation	Cause	Solution
Run taking longer than usual	Buffers are too dilute	Check buffer recipe; re-make if necessary.
	Upper Buffer Chamber is leaking	Make sure the buffer core is firmly seated.
		If you are using the Buffer Dam, make sure the dam is properly positioned in the core. If the core gasket is damaged, replace with a fresh gasket.
		Check to ensure that the XCell4 SureLock™ Assembly is in the locked position
	Voltage is set too low	Set correct voltage as listed on page 13.
When running multiple gels, the total current requirement exceeds the maximum output limit of the power supply	Run fewer gels or use a power supply with higher power output.	
Current reading on power supply is zero or very low	Tape left on the bottom of the cassette	Remove tape from the bottom of the cassette.
	Connection to power supply not complete	Check all connections with a voltmeter for conductance.
	Insufficient buffer level	Make sure the buffer in the Upper Buffer Chamber (cathode) is covering the wells. Be sure there is sufficient buffer (up to the fill line) in the Lower Buffer Chamber.
	Buffer is leaking from the Upper Buffer Chamber	If the level of running buffer drops, the electrophoresis core and cassettes are not properly seated. Repeat the assembly (see page 7). Check to ensure the XCell4 SureLock™ Assembly is in the locked position.
Run is faster than normal with poor resolution	Buffers are too concentrated or incorrect	Check buffer recipe; dilute or re-make if necessary.
	Voltage, current, or wattage is set at a higher limit	Decrease power conditions to the recommended run conditions (page 13).

Appendix

Safety information

Safety

During operation, use the XCell4 SureLock™ Midi-Cell with an external DC power supply designed specifically for electrophoresis applications. This power supply must be isolated from ground so that the DC output is floating and must be equipped with No Load or Open Circuit protection. The maximum electrical operating parameters for the XCell4 SureLock™ Midi-Cell are:

Maximum voltage limit: 600 Volts DC

Maximum power limit: 200 Watts

Operating temperature: 4°C to 40°C

The XCell4 SureLock™ Midi-Cell lid is designed such that if the lid is removed, the electrical connection to the Midi-Cell will be broken. **Do not attempt to use the cell without the cell lid.**

The XCell4 SureLock™ Midi-Cell is designed to meet EN61010-1 Safety Standards. This product is safe to use when operated in accordance with this instruction manual. If this Midi-Cell is used or modified in a manner not specified in this manual, then protection afforded by the Midi-Cell will be impaired. Alteration of this Midi-Cell will:

- Void the warranty.
- Void the EN61010-1 safety standard certification.
- Create a potential safety hazard.

Life Technologies is not responsible for any injury or damage caused by use of this Midi-Cell when operated for purposes which it is not intended. All repairs and service should be performed by Life Technologies.

The XCell4 SureLock™ Midi-Cell is classified as Class II for protection against electrical shock.

Continued on next page

Safety information, continued

Important

The XCell4 SureLock™ Midi-Cell is manufactured in the USA from domestic and International components.

To ensure safe, reliable operation, always operate the XCell4 SureLock™ Midi-Cell according to instructions provided in this manual. Wear protective gloves and safety glasses when working in a laboratory environment.

Life Technologies is not responsible for injuries or damages caused by improper use.

Informational symbols

The symbols used on the XCell4 SureLock™ Midi-Cell are explained below:



Used on the XCell4 SureLock™ Midi-Cell to indicate an area where a potential shock hazard may exist.



Used on the XCell4 SureLock™ Midi-Cell to indicate a warning. The operator's manual should be consulted to avoid possible personal injury or instrument damage.



WEEE (Waste Electrical and Electronic Equipment) symbol indicates that this product should not be disposed of in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE.

Sicherheits-Anweisungen

Sicherheits-Anweisungen

Die XCell4™ Elektrophorese-Zelle muss mit einer geerdeten Gleichstrom-Spannungsquelle betrieben werden, die speziell für die Elektrophorese entwickelt wurde. Um eine Gefährdung des Benutzers durch Stromschlag zu minimieren, muss das Gleichstromversorgungsgerät Gleichstromhochspannung produzieren, die vom Erdleiter elektrisch getrennt ist, und es muss mit einer Schutzschaltung gegen Fehlströme (FI - Schutzschalter) ausgestattet sein. Desweiteren sollten folgende Laufbedingungen für die XCell4™ Elektrophorese-Zelle eingehalten werden:

Maximale Spannung: 600 Volts DC

Maximale Leistung: 200 Watts

Temperatur: 4°C to 40°C

Beim Entfernen des Kammerdeckels der XCell4™ Electrophorese-Zelle wird der Stromflub unterbrochen. Die Benutzung der Zelle ohne Deckel ist verboten.

Die XCell4™ Elektrophorese-Zelle entspricht den Sicherheits-Richtlinien EN 61010-1. Es besteht keinerlei Gefährdung für den Benutzer, wenn die Richtlinien der Gebrauchsanweisung eingehalten werden. Wird die Elektrophorese-Zelle in einer Art und Weise benutzt bzw. modifiziert, die nicht in der Gebrauchsanleitung beschrieben ist, werden die Sicherheitvorkehrungen zum Schutz des Betreibers beeinträchtigt. Bei unsachgemässer Handhabung entfallen alle Garantieansprüche und Sicherheitszulassungen (EN61010-1).

Life Technologies haftet nicht für Verletzungen oder Schäden jeglicher Art, wenn die Elektrophorese-Zelle für Anwendungen eingesetzt wird, für die die Zelle nicht entwickelt wurde. Alle Reparaturen und Wartungen müssen von Life Technologies oder einem von Life Technologies autorisierten Händler vorgenommen werden.

Das Gehäuse der XCell4™ Elektrophorese-Zelle besteht aus Kunststoff und enthält keine leitenden Teile.

Symbolerklärung



Weist auf eine Zone hin, wo Gefährdung durch Stromschlag besteht.



Lesen Sie die Bedienungsanleitung, um Verletzungen oder Beschädigung des Gerätes zu vermeiden.



WEEE

Instructions de sécurité

Instructions de sécurité

La XCell4 SureLock™ Midi-Cell doit être utilisée uniquement avec une alimentation en courant continu DC externe et spécialement conçue pour en usage avec un système électrophorèse. Cette alimentation doit être isolée et sans contact avec la terre et doit être équipée avec un système de protection en cas de Non Charge ou Circuit ouvert.

Le domaine d'utilisation de la XCell4 SureLock™ Midi-Cell est limité par les paramètres de fonctionnement suivants:

Tension maximum: 600 Volts DC

Puissance maximum: 200 Watts

Température: 4°C to 40°C

La XCell4 SureLock™ Midi-Cell est conçue de façon à ce que lorsque le couvercle de la cuve est ouvert, la connexion électrique est interrompue. Il ne faut en aucun cas essayer de faire fonctionner la cuve sans le couvercle.

La XCell4 SureLock™ Midi-Cell satisfait aux normes de sécurité EN 61010-1. La sécurité est assurée dans tous les cas d'une utilisation conforme au manuel d'utilisation. Dans le cas où la cellule serait modifiée ou utilisée de manière non spécifiée dans le manuel d'utilisation, la sécurité de l'utilisateur pourrait être remise en cause. En cas d'utilisation non conforme toutes les garanties ainsi que les certifications de conformité aux normes de sécurité sont abrogées.

La responsabilité de Life Technologies ne peut être mise en cause en cas d'accident et dommages causés par une utilisation non conforme de la XCell4 SureLock™ Midi-Cell. Toute intervention de service ou de réparation doit être uniquement effectuée par Life Technologies ou par un agent agréé de Life Technologies.

La XCell4 SureLock™ Midi-Cell est classifié par rapport à la norme d'isolation électrique en classe II.

Signification des pictogrammes



Sur le XCell4 SureLock™ Midi-Cell, signale les zones où un risque de choc électrique peut exister.



Sur XCell4 SureLock™ Midi-Cell, signale un risque potentiel pour l'utilisateur ou pour l'équipement. Veuillez consulter le mode d'emploi.



WEEE



Product specifications

XCell4 SureLock™ Midi-Cell specifications

Dimensions:	7.5 (l) × 8.35 (w) × 6.3 (h) inches
Upper Buffer Chamber volume:	175 mL in each upper buffer chamber
Lower Buffer Chamber volume:	700 mL with 4 gels
Lower Buffer Chamber material:	Polycarbonate
Buffer Core Material:	Polycarbonate, gold plated copper, platinum, plasticized silicone
Buffer Dam/Displacement Dam Material:	High density polyethylene
Electrode wire:	Platinum (0.010" diameter)
Electrical limits:	600 VDC or 200 Watts
Operating temperature:	4°C to 40°C

The XCell4 SureLock™ Midi-Cell is impervious to alcohol, but not compatible with chlorinated hydrocarbons (e.g. chloroform), aromatic hydrocarbons (e.g. toluene, benzene) or acetone.



The CE mark symbolizes that the product conforms to all applicable European Community provisions for which this marking is required. Operation of the XCell4 SureLock™ Midi-Cell is subject to the conditions described in this manual.

The protection provided by the equipment may be impaired if the equipment is used in a manner not specified by Life Technologies.

Gel specifications

The XCell4 SureLock™ Midi-Cell is compatible with NuPAGE® Novex® and Tris-Glycine Midi precast gels. The Novex® Midi precast gels have the following specifications:

Cassette:	15 cm × 10.3 cm
Gel Size:	13 cm × 8.3 cm
Thickness:	1.0 mm

Sample well capacity

The maximum recommended sample volume/well for different Novex® Midi precast gels is listed below.

Gel thickness	12+2 well	20 well	26 well
1.0 mm	45 µL + 15 µL marker	25 µL	15 µL

Maintenance

Wash the XCell4 SureLock™ Midi-Cell with a mild detergent and rinse with deionized water after each use.

Accessory products

Additional products Additional reagents that may be used for electrophoresis of proteins are available separately from Life Technologies. Ordering information is provided below. For details, visit www.lifetechnologies.com or contact Technical Support (page 23).
A large variety of Novex® Midi Gels and protein standards are available for use with the Midi-Cell, see www.lifetechnologies.com for details.

Midi-Cell spare parts	Quantity	Catalog no.
XCell4 SureLock™ Midi-Cell Lid	1 each	WR0101
XCell4 SureLock™ Midi-Cell Lower Buffer Chamber	1 each	WR0102
XCell4 SureLock™ Midi-Cell Displacement Dam	1 each	WR0106
XCell4 SureLock™ Midi-Cell Wire	1 each	WR0107
Pre-mixed buffers	Quantity	Catalog no.
NuPAGE® LDS Sample Buffer (4X)	10 mL	NP0007
NuPAGE® Sample Reducing Agent (10X)	250 µL	NP0004
	10 mL	NP0009
NuPAGE® Antioxidant	15 mL	NP0005
NuPAGE® MOPS SDS Running Buffer (20X)	500 mL	NP0001
NuPAGE® MES SDS Running Buffer (20X)	500 mL	NP0002
NuPAGE® Tris-Acetate SDS Running Buffer (20X)	500 mL	LA0041
Novex® Tris-Glycine Native Running Buffer (10X)	500 mL	LC2672
Novex® Tris-Glycine Native Sample Buffer (2X)	20 mL	LC2673
Novex® Tris-Glycine SDS Running Buffer (10X)	500 mL	LC2675
Novex® Tris-Glycine SDS Sample Buffer (2X)	20 mL	LC2676
Pipette tips	Quantity	Catalog no.
Gel Loading Tips (Standard Round)	200/pk	LC1001
Flat Gel Loading Tips	200/pk	LC1002
Gel Loading Tips (Eppendorf Round)	200/pk	LC1010
Power supply	Quantity	Catalog no.
ZOOM® Dual Power Supply (100–120 VAC, 50/60 Hz)	1 each	ZP10001
ZOOM® Dual Power Supply (220–240 VAC, 50/60 Hz)	1 each	ZP10002
PowerEase® 90W Power Supply (115/230V~, 50/60Hz)	1 each	PS0090
PowerEase® 90W Power Supply (115/230V~, 50/60Hz)	1 each	PS0091
PowerEase® 300W Power Supply (115/230V~, 50/60Hz)	1 each	PS0300
PowerEase® 300W Power Supply (115/230V~, 50/60Hz)	1 each	PS03001
Novex® Power Supply Adapters	1 set	ZA10001

Technical support

Obtaining Support For the latest services and support information for all locations, go to www.lifetechnologies.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS) Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support.

Certificate of Analysis The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on the Life Technologies web site at www.lifetechnologies.com/termsandconditions. If you have any questions, contact Life Technologies.

For support visit lifetechnologies.com/support or email techsupport@lifetech.com

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