DireCtQuant ELM USER GUIDE

Protein electrophoresis and Western blotting system

For denaturing and reducing polyacrylamide gel electrophoresis

Ultrafast

For Research Use Only

Not Intended for any Animal or Human Diagnostic or Therapeutic Use

www.directquant.eu

Contents

ABOUT Dire*Ct*Quant ELM

Dire*Ct*Quant ELM is a protein electrophoresis and Western blotting system which includes set of reagents designed to achieve superior resolution and shorter running time. It is a significant improvement over widely used Laemmli system. This system can be used only for denaturing and reducing polyacrylamide gel electrophoresis. ELM is specially designed to be used only with samples extracted with **Dire*Ct*Quant 100ST** or **Dire*Ct*Quant 100W**

The main advantages, over the commonly used Laemmli protocol, are:

- Ultrafast (less than 10min) electrophoresis and transfer using standard electrophoresis and transfer equipment;
- Far superior resolution compared with the Laemmli system;
- Tracking dye which does not interfere with fluorescent detection;
- Extremely efficient transfer without toxic components (such as methanol);
- Single buffer required for gel casting;

In addition **Dire*Ct*Quant ELM** is compatible with most commercially available precast Polyacrylamide gels *

* Tested with TGX precast gels (Bio-Rad) and Novex Tris-Glycine gels (ThermoFisher Scientific)

One set of **Dire*Ct*Quant ELM** provides the necessary reagents for at least 10 complete mini (10 cm x 10 cm gels) Western blots using semi-dry transfer.

Individual components can be purchased separately to accommodate your particular need and equipment:
Ref. ELML 5X Loading buffer 3 x 1 ml
Ref. ELMR 10X Running buffer 1500 ml
Ref. ELMT 25X Transfer buffer 6 x 20 ml

SHIPMENT AND STORAGE

The running buffer (ELMR, 10X) and the transfer buffer (ELMT, 25X) are shipped and stored at room temperature.

The loading buffer (ELML, 5X) is shipped at ambient temperature. Store at -20°C up to indicated expiry date or at 4°C up to two months.

Bring all the reagents to room temperature and mix well before use.

COMPONENTS AND EQUIPMENT TO BE PROVIDED BY THE USER

- Electrophoresis system capable of accommodating your gels
- Precast gels or:
  - Gel casting system,
  - Acrylamide:Bis-Acrylamide 39:1 stock solution of 30%
  - APS (Ammonium persulfate) prepare daily at 50 mg/ml in water
  - TEMED (N,N,N',N'-Tetramethylethylenediamine)
- Semi-Dry or Wet Transfer blot system
- Blotting paper with thickness of 3-4mm (or thinner which should be used as stacks to the indicated thickness)
- PVDF membrane
- Molecular biology grade distilled water

! **IMPORTANT:** Carefully check the maximum safety limits (voltage, current and power) of your electrophoretic equipment (power supply, electrophoretic chamber and transfer equipment). Do not use this system with equipment that cannot safely provide the conditions listed in this protocol. Do not combine this system with reagents from another system (running, transfer or loading buffers or with samples extracted with solubilisation buffers other than **DireCtQuant 100ST** or **DireCtQuant 100W**).

Not following these precautions can result in poor performance and could lead to equipment failure and/or personal injury!

GEL PREPARATION

If using precast gels, go to **sample preparation** section.

Resolving gel

Choose the gel percentage depending on the desired separation range (see **Table 1**).

Resolving gel percentage	5%	7,5%	10%	12,5%	15%
MW resolving range	75-350kDa	50-300kDa	25-250kDa	15-200kDa	10-150kDa
Water	7.12 ml	6.28 ml	5.45 ml	4.61 ml	3.78 ml
Acryl amide/Bis-Acrylamyde 30%	1.67 ml	2.50 ml	3.33 ml	4.17 ml	5.00 ml
ELM Running buffer 10X	1 ml	1 ml	1 ml	1 ml	1 ml
APS 50 mg/ml in water	200 µl	200 µl	200 µl	200 µl	200 µl
TEMED	20 µl	20 µl	20 µl	20 µl	20 µl

Table 1. *Recipe for the preparation of one 10cm x 10cm x 1mm (**10ml**) resolving gel.*

Multiply the numbers in the table by the numbers of gels required.

1. **Mix well and pour in the preassembled gel casting system leaving space (at least 5mm form the bottom of the comb) for the stacking gel.**

2. **Cover with layer of 1X ELM running buffer.**

3. **After complete polymerization remove the 1XELM running buffer**

Stacking gel

Stacking gel percentage	4%
Water	3.72 ml
Acrylamide/Bis-Acrylamyde 30%	667 µl
ELM Running buffer 10X	500 µl
APS 5% in water	100 µl
TEMED	10 µl

Table 2. *Recipe for the preparation of one (**5 ml**) stacking gel. Multiply the numbers in the table by the numbers of gels required. To be used with all gels listed in Table 1.*

1. **Mix and pour over the resolving gel. Insert the comb and leave to polymerize completely.**
   * **NOTE:** *If required you can store the gels submerged in 1X ELM Running buffer for several days at 4°C*

SAMPLE PREPARATION

1. **Remove the ELM Loading buffer from the freezer and bring to room temperature.**
   * **NOTE:** *The buffer is stable for at least ten cycles of freeze/thawing*

2. **Mix 10 µl of ELM Loading buffer 5X with 39 µl of sample prepared in DireCtQuant 100ST or DireCtQuant 100W**

3. **Add 1 µl of a reducing agent of your choice (2-mercaptoethanol, 1M dithiothreitol or 0.5M TCEP).**

   * **NOTE:** ***2-Mercaptoethanol/β-mercaptoethanol (2-ME)*** *is usually supplied as a 14M solution (pure liquid) which will result in 360 mM final concentration if 1 µl is used for the sample preparation.* ***Dithiothreitol (DTT)*** *should be freshly prepared as a 1M solution in water (the resulting final concentration will be 25 mM).* ***Tris(2-carboxyethyl)phosphine) (TCEP)*** *should be prepared as a neutral pH solution. The stock is stable at room temperature (the resulting final concentration will be 12,5mM). We recommend the use of TCEP, as it is without-odour, non volatile and irreversible reducing agent.*

4. **Incubate at 90°C for 3min preferably with shaking**
   ! **CAUTION:** *Use appropriate protection when handling hot tubes.*

   * **NOTE:** *The buffer composition provides a complete reduction of at least 20 µg total proteins per sample*

5. **Leave the sample to cool down to room temperature**

6. **Spin the sample to collect it to the bottom of the tube and proceed with the gel electrophoresis.**

GEL SETUP AND ELECTROPHORESIS

1. **To prepare 1 litre of 1X Running buffer mix 100 ml of 10X ELM Running buffer with 900 ml distilled water and mix well.**
   * **NOTE:** *If using precast gels assemble the electrophoresis system and the precast gel as recommended by the manufacturer. Use ONLY the 1X ELM Running buffers to fill the cuvette and to rinse the sample wells.*

* **NOTE:** *Use at least 200ml of 1X buffer per gels but not less than the minimum specified for your electrophoresis equipment*

2. **Load samples on the gel**

3. **Attach the cables and run the electrophoresis applying up to 600V per mini-gel**

   ! **CAUTION:** *Check carefully the voltage limit and current/power limit of your electrophoresis system and power supply. Never exceed the recommended safety parameters of your equipment! In order to prevent damages caused by the short circuits always set up the indicated current or power limit of the equipment on the power supply!*

   * **NOTE:** *The expected current per 10 cm gel of 1mm thickness is not more than 60mA. No significant warm up (no more than 40°C) is expected at the end of the electrophoresis.*

   * **NOTE:** *If 600V per mini-gel used the separation will be completed in* ***about 10 min*** *visualized by the escape of the red tracking dye from the gel. You can follow the separation in real time if a pre-stained molecular weight marker is included.*

4. **Disassemble the electrophoresis tank, remove the gel from the gel cassette and prepare it for transfer by submerging it in 1X transfer buffer for 5 to 10 min.**

TRANSFER

1. **Prepare enough transfer buffer by diluting the supplied 25X Transfer buffer with distilled water (for example mix 2ml of 25X ELMT with 48ml of water).**

   * **NOTE:** *At least 50ml transfer buffer are needed per mini-gel for semi-dry transfer.*

2. **Activate a piece of PVDF membrane by submerging in 96-100% ethanol for 10-20s with shaking followed by 1-2min incubation in transfer buffer**

   * **NOTE:** *The PVDF membrane should be at least 2mm larger than the gel in all directions.*

3. **Prepare a transfer sandwich in the following order:**
   - **Cathode side (-)**
   - **Approx. 3mm blotting paper (or paper stack) pre-wetted with transfer buffer**
   - **Gel**
   - **PVDF membrane**
   - **Approx. 3mm blotting paper (or paper stack) pre-wetted with transfer**
   - **Anode side (+)**

4. **Assemble the semi-dry transfer cell.**
   In case of wet-transfer system is used, fill the chamber with 1X Transfer buffer to the indicated level

5. **Run the semi-dry transfer at 25V for 10 min.**
   For wet transfer use up to 30V per cm distance between the electrodes for 10 min (see example)

   ! **CAUTION:** *Always set the current/power limit on the power supply at indicated level, to prevent damages caused by the short circuits!*

   ❖ **EXAMPLE:** *For Bio-Rad Mini-Protean system where the distance between the electrodes is 4 cm the current should be set to 120V*

6. **Disassemble the transfer sandwich and mark the side of the membrane which was in contact with the gel.**

   * **NOTE:** **DireCtQuant EML** *system provides far superior binding of the separated protein to the membrane compared with the traditional transfer. As a result the majority of the proteins are bound to the surface of the membrane touching the gel. If during the following detection steps this side is preferentially exposed to the detection reagents this will results in more efficient detection.*

7. **Rinse the membrane in 1X transfer buffer to remove possible adhered gel pieces and wash in at least 50 ml distilled water for 1min with agitation**

8. **Follow with the protein immunodetection as required by your research project or dry the membrane for storage.**

GENERAL STATEMENT

Handle our products in accordance with safe laboratory practices:
***Wear* suitable *protective gloves, eyewear* and *clothing*.**

Please read and understand the MSDS data provided and updated at directquant.eu

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In case of any doubt please feel free to contact DireCtQuant team at:
customersupport@directquant.eu