Tank Blotting Protocol

Using the Mini Trans-Blot® Cell

- Prepare 1 L 1x transfer buffer by diluting 100 ml 10x Tris/glycine premixed buffer (catalog #161-0734) with 700 ml water and 200 ml methanol
- Rinse gels briefly in water and equilibrate in 1x transfer buffer for 15 min
- Soak 2 pieces of filter paper (the same size as the gel), 2 foam pads, and nitrocellulose membranes in 1x transfer buffer until wet; if PVDF is used, activate the PVDF by soaking in 100% methanol briefly, then transfer to transfer buffer
- Open the cassette and place in a tray filled with transfer buffer; place a foam pad on the black side of the cassette
- Place a piece of filter paper on top of the foam pad, then carefully place the gel on top of the filter paper; remove bubbles with a roller
- Carefully place the membrane on top of the gel; if possible, do not move the membrane after it is positioned, and roll out any air bubbles
- Place a second piece of filter paper on top of the membrane, remove bubbles with a roller, and place the second foam pad on top of the filter paper
- Close the cassette and insert into the tank (the black side of the cassette should face the black side of the central core)
- Insert frozen cooling unit
- If transferring more than one gel, repeat the above steps with a second cassette
- Add transfer buffer to the tank until the buffer level reaches the upper fill line
- Place the lid on the tank to complete assembly

Recommended Transfer Conditions

Method	Standard Condition	Rapid Condition
Tank blotting	100 V, 30–60 min	150 V, 15–30 min*
Semi-dry	15–25 V, 15–30 min	

For more information, refer to the Bio-Rad Protein Blotting Guide (bulletin 2895).

* This process is protein dependent.

Semi-Dry Transfer Cell Protocol

Using the Trans-Blot® SD Semi-Dry Transfer Cell

- Prepare ~1 L of a 1x transfer buffer solution by diluting 100 ml 10x Tris/glycine premixed buffer (catalog #161-0734) with 700 ml water and 200 ml methanol
- Rinse gel briefly in water and equilibrate in 1x transfer buffer for 15 min
- Soak 2 pieces of precut extra thick filter paper (match the size of the gel) and nitrocellulose membrane in transfer buffer until wet; if PVDF is used, activate the PVDF by soaking in 100% methanol briefly, then equilibrate in transfer buffer
- Place 1 filter paper on the anode side of the semi-dry apparatus
- Place membrane (PVDF or nitrocellulose) on top of the filter paper
- Carefully place gel on top of the membrane
- Place the second piece of filter paper on top of the gel; roll out any bubbles that may have formed between the stacks
- Carefully place the cathode assembly onto the transfer stack and then place the safety cover back onto the unit

Note: If using the Trans-Blot Turbo semi-dry transfer system and transfer packs, see bulletins 10016505 and 10019593.

10026447 Rev C US/EG *13-2049 1113*

Mini-PROTEAN® TGX™ Precast Gels

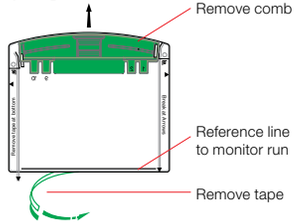
Quick Start Guide

BIO-RAD

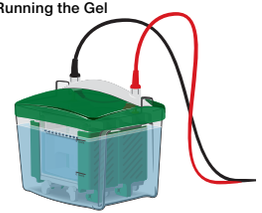
Instructions for Using Mini-PROTEAN® Precast Gels

1. **Remove Comb:** Position thumb on indentation (middle of comb) and remove comb by pulling upward in one smooth motion.
2. **Remove Tape:** Pull the green tape gently to remove it from the bottom of the cassette.
3. **Assemble Mini-PROTEAN Tetra Cell:** Assemble the cassette into the running module of the Mini-PROTEAN Tetra system. Add running buffer to the inner and outer chambers. Use a syringe or a disposable transfer pipet to rinse the wells with running buffer.
4. **Run Gel:** Prepare the samples and load into the wells. If using Bio-Rad Precision Plus Protein™ standards, load 10 µl (5 µl for Precision Plus Protein™ WesternC™ standards). Run the gel until the dye front reaches the reference line. Refer to the instruction manual (#1658100) for more information on running conditions. At the completion of the run, disconnect the cell and remove the cassette.
5. **Open Cassette:** Align the arrow on the opening lever (catalog #456-0000) with the arrows marked on the cassette. Insert the lever between the cassette plates at the indicated locations and apply downward pressure to break the seal. Gently pull apart the two plates beginning from the top of the cassette.
6. **Remove Gel:** Gently remove the gel from the cassette. **Note:** If using Mini-PROTEAN® TGX Stain-Free™ gels, gels should be activated in a stain-free enabled imager (Gel Doc™ EZ or ChemiDoc™ MP) at this point.

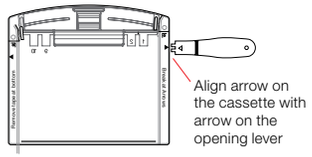
Preparing the Cassette



Running the Gel



Opening the Cassette



Note: Opening lever is 100% aluminum and recyclable.

Instructions are provided for electrophoresis of Mini-PROTEAN TGX long shelf life precast gels using the Mini-PROTEAN Tetra cell system.

	Reagent	Reduced Sample	Non-Reduced Sample
Prepare samples	Sample	5 µl	5 µl
	2x Laemmli sample buffer (catalog #161-0737)*	4.75 µl	5 µl
	β-mercaptoethanol** (catalog #161-0710)	0.25 µl	—
	Total volume	10 µl	10 µl
	Heat samples at 90–100°C for 5 min		
Prepare running buffer	Prepare 1x Laemmli SDS-PAGE running buffer by adding 100 ml 10x TGS running buffer (catalog #161-0732) to 900 ml deionized water.		
Load running buffer	Remove the comb and tape from the bottom of the gel as described on page 2 and assemble the Mini-PROTEAN Tetra cell. Fill the upper (inner) buffer chamber of each core with 200 ml 1x TGS running buffer. Fill the lower (outer) buffer chamber as indicated on the Running Conditions table.		
Load sample	Load the appropriate volume of your protein sample on the gel and run the gel.		

* Note: 4x Laemmli sample buffer also available (catalog # 161-0747). See manual for instructions on use.

** DTT may also be used as a reducing agent. If so, add DTT to a concentration of 100 mM in 2x Laemmli sample buffer. Mix the sample with 2x reducing Laemmli sample buffer at a 1:1 ratio.

	100 V Low Voltage	200 V Standard	300 V Rapid 1	400 V Rapid 2
Run time	85–95 min	30–40 min	15–20 min	10–15 min
Expected current (per gel)				
Initial:	15–20 mA	25–50 mA	55–75 mA	89–140 mA
Final:	5–10 mA	20–31 mA	45–70 mA	81–127 mA
Expected temperature	25°C	25–35°C	30–45°C	40–55°C
Lower buffer volume (for 2 gels)	550 ml	550 ml	800 ml	800 ml
Lower buffer volume (for 4 gels)	800 ml	800 ml	800 ml	800 ml

Note: 1. When running only 1–2 gels in the Mini-PROTEAN Tetra cell, do not leave the companion module in the tank.

2. Do not run different gel types (chemistries) or percentages at the same time.

For detailed instructions, refer to the Mini-PROTEAN Precast Gels Instruction Manual and Application Guide (bulletin 1658100), available at **www.bio-rad.com**, or contact Technical Support at lsg_techserv_us@bio-rad.com or at 1-800-424-6723.

BIO-RAD