G-Blot for
High Throughput Western Blotting

User Manual

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1. Introduction

Overview

In the G-Blot system, the HT-1 Horizontal Tank is designed to be used with G96 and G48 Precast Gels to conduct high-throughput western blots. The tank is used for electrophoresis; transfer must be performed on a separate device.

The tank components include the tank body, the lid, and wires to connect to a power supply. Always affix the lid to the tank before plugging the power wires in.

Specifications

HT-1 Horizontal Tank

Tank dimensions: 217 mm * 172 mm * 162 mm

Weight: 504 g

Tank Material: PETG

Electrode Wires: Platinum

Electrode Prongs: Nickel-finished brass

Hex nuts: nylon

Chemical Compatibility: NOT compatible with alcohols, acetaldehyde, strong/concentrated acids or bases, aromatic hydrocarbons (e.g., benzene, toluene), halogenated hydrocarbons, esters, ketones (e.g., acetone), and oxidizing agents. Usage of incompatible chemicals will void all warranties.

Temperature limit: 4 °C to 50 °C

Voltage limit: 150 V

Power limit: 100 W

Buffer Volume: 600 mL

2. Safety

Read all safety guidelines before using the instrument.

- This system is meant for research purposes only.
- Do not alter or modify the tank in any way.
- Always use PPE when handling the tank and performing experiments, including but not limited to gloves, safety glasses, and lab coats.
- Read and understand Safety Data Sheets (SDSs) for all materials prior to use.
- Handle hazardous materials in a fume hood.
- Use the tank on a clean, level, and dry workstation.
- Do not move the tank during operation.

- Always affix the lid to the tank before inserting the power wires into the power supply.
- Ensure electrode wires are secure and fully pressed into the electrode prongs prior to supplying power.
- Use an external DC voltage power supply with properly configured and approved cords. The power supply must always be properly grounded.
- Do not exceed 150 V or 100 Watts of power.
- Turn off the power supply before removing the wires from the tank.
- Remove the power cords and lid from the tank before handling its contents.
- The temperature range for operation is 4 °C to 50 °C.
- If temperature exceeds 50 °C, turn off the power supply, allow the system to cool, and end the experiment.
- Clean the tank and lid after each use (see maintenance section).
- Inspect the tank prior to use and contact support if there are any issues.

3. Setup

Inspect the tank for any damage, particularly to the wiring. If the tank is damaged, please contact support.

You may practice assembling an empty tank before your first experiment. Line up the lid with the tank so that electrodes from the tank go through the holes in the lid. Gently press down the lid until it is resting on the tank. To remove the lid, lift it with its handles.

4. Electrophoresis

Running Buffer

Recipe for 1 L 10X (1 M) and 1X (0.1 M) Tris-HCl Running buffer. Scale as desired.

Reagents

- Tris base
- Concentrated HCl (~37%)
- 10% (10 g / 100 mL) sodium dodecyl sulfate (SDS)
- ddH_2O

Procedure (10X)

1. Tare a balance with a ~2 L glass beaker.
2. Weigh 121.1 g Tris base in the beaker.
3. Add ~500 mL ddH_2O to the beaker.
4. Add a magnetic stir bar and place on a stir plate with gentle agitation and pH monitoring.
    - The pH should be ~11 once the Tris has dissolved.
5. After the Tris has dissolved, add concentrated HCl slowly until pH = 7.5.
6. Transfer to a 1 L graduated cylinder and add ddH_2O to 900 mL.
7. Add 100 mL 10% SDS.
8. Store at room temperature.

Procedure (1X)

1. Place 100 mL of 10X buffer from above in a 1 L graduated cylinder.
2. Add 900 mL of ddH_2O.
3. It should be stored at 4 ^oC for electrophoresis.

Electrophoresis Procedure

Operation of the horizontal tank for SDS-PAGE.

Reagents

- Cold (4 °C) 1X Tris-HCl running buffer
- Samples (standard preparation for SDS-PAGE in Laemmli Buffer)

Procedure

1. Ensure 1X Tris-HCl running buffer is cold (4 °C).
2. Rinse the tank and lid with cold water if it was not cleaned after the last use. Hot water may deform the plastic.
3. Place the tank on a level surface.
4. Remove the pre-cast gel from the bag, take off the lid, carefully re-center the gel on the base if needed, and place the gel and base into the center tray of the tank.
    - If the gel has expanded such that it exceeds its base and contacts both sides of the tank, then discard it or trim the edges.
5. Fill the tank slowly with ~600 mL of cold (4 °C) running buffer.
    - There should be ~0.5 inches (~1.25 cm) of buffer above the gel.
    - This volume of buffer will reach the fill line on the tank.
6. Load samples in wells.
    - Each well is 56 µL; we recommend loading 10 – 25 µL.
    - Single channel or 12-channel micropipettes may be used.
    - Having bromophenol blue in your sample is generally recommended.
7. Affix the lid to the tank.
8. Connect the tank to a power supply. Protein samples will migrate towards the red wire.
9. Refer to the table below for our recommended voltage and run time settings based on the type of gel you are using. These are intended as starting points. Currents are usually ~200 - 300 mA and may increase during electrophoresis.

	6% Acrylamide	*10% Acrylamide*
G96	50 V, 60 min	50 V, 60 min
G48	50 V, 90 min	50 V, 120 min

10. Settings may need adjustment depending on your sample type, buffer conditions, and experimental goals.

11. Monitor the progress of the run periodically.
12. We generally recommend stopping the run when bromophenol blue dye reaches the beginning of the next row of wells.
13. Once electrophoresis is complete, turn off the power supply, unplug the electrodes, detach the lid from the tank, and lift the gel out from the tank by its base. The gel is ready for subsequent processing or analysis.
14. Rinse the tank and lid with cold water and allow it to air dry.

5. Wet Transfer

Transfer Buffer

Recipe for 800 mL 10X and 2L 1X Tris-Glycine transfer buffer. Scale as desired.

Reagents

- Tris base
- Glycine
- Methanol
- ddH_2O

Procedure (10X)

1. Tare a balance with a ~2 L glass beaker.
2. Weigh 30.3 g Tris base in the beaker.
3. Weigh 144 g Glycine in the beaker.
4. Add ~500 mL ddH_2O to the beaker.
5. Add a magnetic stir bar and place on a stir plate with gentle agitation.
    - Solution can take several hours to dissolve. Gentle heat can help.
6. Transfer to a 1L graduated cylinder and add ddH_2O to 800 mL.
7. Store at room temperature.

Procedure (1X–made fresh the day before transfer)

1. Place 160 mL of 10X buffer from above in a 2 L graduated cylinder.
2. Add 400 mL of methanol.
3. Add ddH_2O to 2000 mL.
4. Store at 4 ºC for transfer.

Transfer Procedure

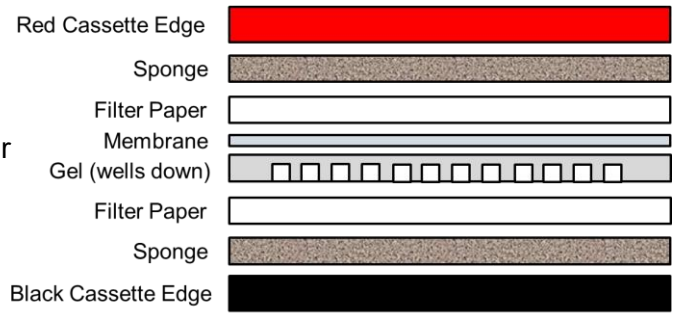
Wet transfer after horizontal SDS-PAGE.

Reagents

- Cold (4°C) 1X transfer buffer
- Nitrocellulose or PVDF membrane
- Methanol (if using PVDF membrane)
- Blotter paper
- Transfer tank and accessories

Procedure

1. With the gel on the gel base, soak it in cold transfer buffer for 5 minutes, wells up.
2. While soaking, place a cold transfer buffer-soaked sponge (from kit) onto the transfer cassette (black side down–also from kit). Fill this chamber with ample transfer buffer.
3. Place a cold transfer buffer-soaked piece of blotter paper slowly onto the top of the gel while the gel remains submerged. The paper should cover the gel wells.
4. Lift the gel base/gel/filter paper assembly, flip it to carefully remove the gel base, and then place the gel with filter paper onto the sponge, filter paper side down.
5. Adjust the gel position so that subsequent cassette clamping does not damage gel.
6. Submerge gel in transfer buffer (this improves membrane adhesion).
7. Carefully place a cut-to-size membrane onto the gel and gently roll to remove air.
8. Carefully place soaked blotter paper on top of the membrane and gently roll as above.
9. Place a cold transfer buffer-soaked sponge onto the paper.



10. Carefully close and clamp the cassette. The final assembly is pictured.
11. Assemble the transfer tank per the manufacturer's instructions.
12. Transfer for 90 minutes at 500 mA at room temperature or 16 hours, 30 V at 4°C.
    - In limited studies, we have observed more uniform transfer at 4°C.

6. Maintenance and Troubleshooting

Cleaning the Tank

After removing the lid, electrode wires, and the contents of the tank, rinse the tank and lid with cold water to remove residue from the buffer. Hot water may deform the plastic. Allow the tank and lid to air dry prior to storage.

Inspecting and Replacing Components

Ensure that the platinum wiring on both sides of the tank does not have visible damage or gaps. Ensure that there are no gaps or abrasions in the insulation underneath the electrodes outside of the tank. If the tank is damaged, please discontinue use and contact support.

Common Issues and Solutions

Wells or Samples Are Difficult to See or Load

- Shine a light from a side angle to make the wells more visible.
- If samples are hard to see during loading or electrophoresis, put a sheet of white paper underneath the tank for contrast.

Gel Floating

- If the gel has expanded to touch the sides of the tank, trim the short edges of the gel.
- Ensure the running buffer has been cooled to 4 C prior to use.
- The gel may float after electrophoresis has been conducted for a long enough time to raise the temperature of the system. Run electrophoresis in a cold room.

Low or High Voltage or Current During Electrophoresis

- Ensure that the running buffer was made to specification.
- Cool running buffer to 4 C.
- Fill tank with running buffer to the fill line.

Slow Migration

- Ensure that the running buffer was made to specification.
- Fill tank with running buffer to the fill line.
- Increase voltage (if at constant voltage) or current (if at constant current).

Non-uniform Migration

- Reduce voltage or current.
- Allow electrophoresis to progress for a longer period while monitoring it.

Poor Resolution

- Allow electrophoresis to progress for a longer period while monitoring it.

High Signal Variation

- Keep pipetting techniques consistent for all samples loaded.

- Target 10 – 25 µL sample volumes for each well.
- Transfer overnight in a cold room.

Poor Transfer

- Ensure full contact between gel and membrane.
- Ensure that transfer buffer was made to specification.
- Avoid uneven pressure on the gel during transfer.

7. Support

Warranty

The HT-1 Horizontal Tank has a 1 year warranty for defects in manufacturing. Blotting Innovations will repair or replace defective parts during this period (1 year from customer receipt) free of charge.

This warranty does not cover defects from accidents, misuse, improper operation, or incompatible chemicals.

Units that were modified or repaired by anyone other than Blotting Innovations are not valid for warranty replacement or repair.

G96 and G48 Pre-Cast gels have a quality guarantee up to their expiration date (1 year from casting date).

Contact Information

For support, please contact Blotting Innovations:

cameron@blottinginnovations.com

135 Watkins St, Suite C

Central SC 29630

8. References

Patents pending: US20230127180A1, WO2023069756A1, EP4419901A1.

Further Reading

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