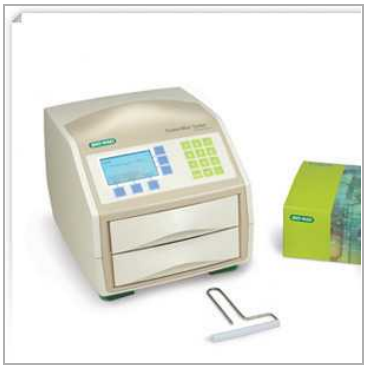

MAR 09, 2023

OPEN ACCESS

Protocol Citation: Lynn Doran, Steven J Burgess 2023. Protein Transfer using Bio-rad TransBlot Turbo with Turbo RTA Transfer Kit. **protocols.io** https://protocols.io/view/protein-transfer-using-bio-rad-transblot-turbo-wit-cquhvwt6

Protocol status: Working
We use this protocol and it's working

Created: Mar 09, 2023

Last Modified: Mar 09, 2023

PROTOCOL integer ID: 78441

Protein Transfer using Bio-rad TransBlot Turbo with Turbo RTA Transfer Kit

Forked from Protein Transfer using Bio-rad TransBlot Turbo

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ABSTRACT

A protocol for transfer of proteins from acrylamide gels onto nitrocellulose membrane for immunoblot analysis. This protocol is based on the assumption that TGX pre-cast gels have been used, the procedure is very similar for self-made gels, just that the running time is longer. Using the transfer packs is economically similar to wet-transfer using MeOH.

Note: Clean the apparatus immediately after use with dH_2O and dry. This prevents the build-up of salts and rust which impair performance.

Literature:
Full manual: https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10020688.pdf
Quick-start: https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10016505D.pdf
Transfer pack: https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10019593D.pdf

IMAGE ATTRIBUTION

Image reproduced from the Bio-rad website

MATERIALS

- TransBlot^{R}-Turbo^{TM} Transfer System (Bio-Rad Laboratories; 1704150)
- Trans-Blot Turbo RTA Mini 0.2 µm Nitrocellulose Transfer Kit, for 40 blots#1704270

1 Wet and equilibrate the membrane.
- Nitrocellulose: 2-3 min in 1X transfer buffer.
- PVDF: Soak in methanol until translucent than transfer to 1X transfer buffer for 2-3 min.

2 Soak transfer stacks of pads (separated by blue paper) side by side in 1X transfer buffer for 2-3 min.

3 Following SDS-PAGE electrophoresis, remove the pre-cast gel by using the small green Bio-rad lever tool to crack apart the cast at each arrow. Gently remove one side of the plastic cast. Use the green Bio-rad scraper to cut off the "arms" that formed the wells and the bottom edge of the gel that was below the junction of the two cast pieces.

4 Using tweezers, move a stack of transfer pads to the cassette. Use the wetted, small roller to smooth the transfer pads.

5 Using tweezers, move the membrane to the center of the transfer pads in the cassette. Use the wetted, small roller to smooth the membrane.

> **Note**
>
> Make sure gloves, tweezers, and roller have all been cleaned with ethanol and dried prior to handling the membrane. Fingerprints or dirt will cause high background noise if transferred to the membrane at this stage.

6 Place the gel in the middle of the membrane and roll to remove air-bubbles.

> **Note**
>
> Any air bubbles between gel and membrane will be visible in final imaging and may obscure protein bands of interest if located over the analysis lanes of the gel.

7 Place the top stack on top of the gel, gently roll

8 Close the cassette lid, taking care not to disturb the gel

9 Place the cassette in the Trans-Blot Turbo and follow the instructions on the machine (Fast protocol TGX gel - 3 minutes).

Equipment	
Trans-Blot® Turbo™	NAME
Protein transfer apparatus	TYPE
Bio-rad Laboratories	BRAND
1704150EDU	SKU
https://www.bio-rad.com/en-us/product/trans-blot-turbo-transfer-system?ID=M7FAXO15	LINK

10 Either dry membrane and store at 4 °C for later use or proceed immediately to fluorescent western protocol