Trans-Blot® Turbo™ Transfer with Home-made buffers

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Abstract

This is our transfer protocol for the transfer of proteins using the Trans-Blot Turbo Transfer system from Bio-Rad. We use it with our single gel we have made based the One-Gel system by Ahn T et al (2001) (DOI:10.1006/abio.2001.5038). It should work the same for Laemmli-based gel systems.

We have achieved transfer from proteins ranging from 270 kDa to 26 kDa present in the same gel.

The Turbo Transfer has been standarized in our lab since October 2016, therefore there are no publications yet using the method. Data using the Ahn et al gel system have been used in the following publications.

Zeitler AF, Gerrer KH, Haas R, Jiménez-Soto LF. Optimized semi-quantitative blot analysis in infection assays using the Stain-Free technology. J Microbiol Methods. 2016 Jul;126:38-41. doi: 10.1016/j.mimet.2016.04.016. PubMed PMID: 27150675.
Jiménez-Soto LF, Haas R. The CagA toxin of Helicobacter pylori: abundant production but relatively low amount translocated. Sci Rep. 2016 Mar 17;6:23227.doi: 10.1038/srep23227. PubMed PMID: 26983895; PubMed Central PMCID: PMC4794710.Jiménez-Soto LF, Clausen S, Sprenger A, Ertl C, Haas R. Dynamics of the Cag-type IV secretion system of Helicobacter pylori as studied by bacterial co-infections. Cell Microbiol. 2013 Nov;15(11):1924-37. doi: 10.1111/cmi.12166. PubMed PMID: 23844976.

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Protocol

Prepare Buffers

Step 1.

Anode buffer I 0,3 M Tris pH 10,4; 10% Methanol
Anode buffer II 25 mM Tris pH 10,4; 10% Methanol
Cathode Buffer 25 mM Tris, 40 mM 6-Amino-n-Caproic acid, 10% Methanol, final pH 9,4

SAFETY INFORMATION
Methanol is toxic. Please protect you from direct contact or inhalation.

ANNOTATIONS

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Sorry. Submerge membrane in **Anode II solution.**

Cut the membrane and paper

Step 2.

We use mini-protean gels (Bio-Rad). Our PVDF (0,22µm) membranes are 8,7 mm x 6,2 mm. The paper should have similar dimensions.

Paper used: Kimberly Clark , WYPALL X60 (KC 6034)

Activate your gel (Only if you are using Stain-Free system)

Step 3.

Activate the membrane in Methanol for 15-30 seconds. Transfer then to Anode II buffer.

Step 4.

After methanol exposure, the PVDF membrane looks "wet". You will see that the membrane retains its highly hidrophobic properties when you transfer it to the Anode I solution. Make sure that to immerse the membrane so long until it does not float on the surface of the buffer. It should remain submerged.

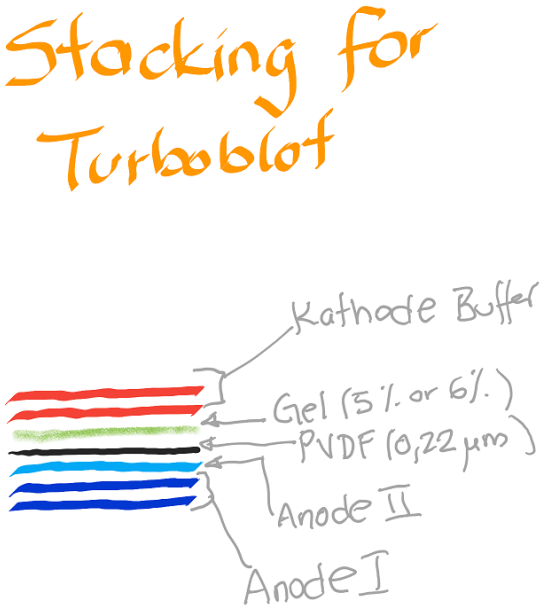
SAFETY INFORMATION

Methanol is toxic. Please protect you from direct contact or inhalation.

Prepare the stack

Step 5.

See picture attached for the order of the paper in the stack. Each line shown for paper, represent a single paper. If you have doubts, measure the thickness of 2 single layer. It should be 1 mm approx.



NOTES

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Make sure you remove ALL bubbles from between the stacks before running using a roller (see pictures attached to step 8)

Start the transfer

Step 6.

We use the following times and conditions for the Turbo Blot transfer machine from Bio-Rad.

20 minutes at 1.3 A, Max. 25V for one mini-gel transfer

20 minutes at 2.5 A - Max. 25V for two minigels transfer.

EXPECTED RESULTS

If your paper, buffers and membranes are working fine, you should have a constant 1,3 A (+/- 0,1) (one gel) of 2,5 A (+/- 0,2) (two gels) for the duration of the transfer.

Remove the membrane

Step 7.

Open up the cassette with the stack and remove carefully the membrane. Place the membrane to dry for 1h at 37°C to bind the proteins to the membrane. Store at 4°C for up to 2 weeks, and at -70°C for up to 6 months. Avoid rubbing the surface of the membrane with the bound proteins.

SAFETY INFORMATION

Be aware that the whole stack of paper and membrane will be hot. The vapors might not be healthy. Protect yourself and those around you from the fumes.

NOTES

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Before you start your western blot, reactivate the membrane with methanol and follow with the blocking solution.

CRITICAL POINT: Make sure that the membrane does not dry at all. For this, it will be ideal if you can activate the membrane with methanol in the same container where you will perform the blocking. In warm temperatures, the methanol evaporates really fast from the membrane causing patches later on.

Information of new users of Trans-blot Turbo system from Bio-Rad

Step 8.

I am attaching pictures of our system to give some spatial orientation for those of you who have not used this system yet and some components you will need.