



Dec 24, 2020

3. LiCor_Blotting_Protocol

Elizabeth Fozo¹¹In-house protocol

1

Works for me

This protocol is published without a DOI.



Eadewunm

ABSTRACT

Blotting Protocol for use on LiCor Odyssey imager.

PROTOCOL CITATION

Elizabeth Fozo 2020. 3. LiCor_Blotting_Protocol. [protocols.io](https://protocols.io/view/3-licor-blotting-protocol-bq2dmya6)
<https://protocols.io/view/3-licor-blotting-protocol-bq2dmya6>

KEYWORDS

LiCor Odessey imaging, LiCor, Protocol, Blotting protocol, imaging

LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Dec 24, 2020

LAST MODIFIED

Dec 24, 2020

PROTOCOL INTEGER ID

45861

DISCLAIMER:

DISCLAIMER: THIS WORK IS IN PROGRESS. IT IS FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer-reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

Blotting Protocol for use on LiCor Odyssey imager.

Blotting Protocol for use on LiCor Odyssey imager.

- 1 Use only the **Licor Immobilon FL** PVDF membranes. Standard PVDF has an unacceptable background signal in the IR. Handle the membranes with forceps at the very edges, and minimize contact with gloves. Damage to the membrane can show up on the image of the blot. **Use only dishes that are clean and have not contained Coomassie blue** – the instrument is very sensitive and will detect traces of blue dye on the blot. The blue dye in the pre-stained markers will be detected, which is convenient. The red marker in the set will not be detected

- 1.1 Place the membrane in a clean dish containing 100% methanol and activate for 30 seconds.
 - 1.2 Wash for 5 seconds with Milli Q water, and then incubate in transfer buffer(standard formulation)at room temperature until ready to use.
 - 1.3 Assemble the cassette for the mini blot module. The transfer is performed in the cold room at 20V for 50 minutes.
 - 1.4 After transfer, the membranes are trimmed to the size of the gel and placed on Whatman paper until completely dry. This fixes the protein onto the membrane and improves signal intensity. Allow a minimum of 1 hour for drying, or they can be left overnight.
- 2 We use the commercial Licor Blocking buffer stock for the IR blots. This gives excellent results with respect to the background. Prepare just what you need for a single day – do not store diluted buffer overnight or you will get an increased background.
- 2.1 When ready to continue processing the blot, activate with 100% methanol for 30 seconds, then wash for 5 seconds wash with Milli Q water.
 - 2.2 Dilute the commercial blocking buffer stock 1:1 with TBS (50% Licor blocking buffer, 50% TBS = **blocking buffer**). Incubate in 10 ml blocking buffer(per square dish) for 1 hour.
 - 2.3 For antibody incubation, the membranes will be incubated in a **blocking buffer supplemented with 0.1% Tween 20 (=Antibody Buffer)**. Primary antibody concentrations are 1:10,000 for both FLAG and 1:50000 for OmpA. Both antibodies are added at the same time. Incubate with primary antibodies for 1 hour.
 - 2.4 Wash the blots 4 X 5' with **TBST wash buffer** (TBS plus 0.1% Tween 20). Note: this is just TBST and does not contain any of the LiCor commercial blocking buffers.
 - 2.5 Incubate with IR dye conjugated secondary antibodies, diluted in **Antibody Buffer** at the following concentrations: IR dye 680 Goat anti-rat, 1:10,000 (detects FLAG Ab) and IR dye 800 Anti-rabbit (ask from Reynold's lab), 1:10,000 (detects OmpA Ab).
 - 2.6 Wash 4 X 5' with **TBST wash buffer** and take for imaging on the LiCor Odyssey instrument.