3. LiCor_Blotting_Protocol

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[1]In-house protocol

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Blotting Protocol for use on LiCor Odyssey imager.

PROTOCOL CITATION

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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 of1 hour for drying, or they can be left overnight.

2 We use the commercialLicorBlocking buffer stock for the IRblots. 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 and1:50000 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.