Buffers

1 L 10x Tris-Glycine-SDS
-30 g Tris Base
-144 g Glycine
-10 g SDS
-ddH_2O to 1 L, store at RT

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-30 g Tris Base
-144 g Glycine
-ddH_2O to 1 L, store at RT

1 L SDS-PAGE running buffer
-100 mL 10x Tris-Glycine-SDS
-ddH_2O to 1 L, cool to 4^oC and store at 4^oC

1 L Transfer Buffer
-100 mL 10x Tris-Glycine
-200 mL Methanol
-ddH_2O to 1 L, cool to 4^oC and store at 4^oC

1 L 10x PBS
-80 g NaCl
-2 g KCl
-14.4 g Na_2HPO_4 (or 27.2 g $Na_2HPO_4 \bullet 7H_2O$)
-2.4 g KH_2PO_4
-ddH_2O to 1 L, store at RT

1 L PBS-Tween
-100 mL 10x PBS
-0.5 mL Tween-20 (use a cut pipette tip)
-ddH_2O to 1 L, mix with a stirbar, store at RT

Protocol

Day 1
-(morning) Load samples (in 1x LDS + BME) into a Tris-glycine polyacrylamide gel
-Run in SDS-PAGE running buffer for approx. 80 min at 180 V limiting (or other parameters depending on the sample)
-Recover gel, remove wells and foot, soak in transfer buffer and place in a transfer sandwich (from bottom to top: black plastic, 2x black sponge, filter paper, gel [inverted], membrane presoaked in transfer buffer, filter paper, 2x white sponge, white plastic)
-Transfer in transfer buffer at 70 V limiting for 2 hrs in the cold room (use an ice block to cool the buffer) or 100 mA limiting overnight. Alternatively, if it's an easy blot do 90V limiting for 1 hr

-During this time, make 40 mL of blocking solution by adding 2 g blocking reagent (Biorad) to 40 mL PBS-tween in a conical and inverting until it dissolves.
-Recover the membrane and cut (if appropriate)
-OPTIONAL: Stain membrane in Ponceau by rocking for 5 min in stain and 1 min in ddH_2O
-Place membrane(s) in 30 mL blocking solution
-Shake for 30 min at room temp
-During this time, add 40 mL PBS-Tween to the remaining 10 mL blocking solution. This is your 1% milk solution
-Make antibody dilutions in Eppis using 1% milk solution
-Recover membranes and place in heat-sealable pouches
-Heat seal so all but one side is sealed
-Add antibody to the open end and seal it. Make sure to label each pouch and keep track if you are doing multiple blots.
-Nutate in the cold room overnight
-Place the rest of the 1% milk solution at 4^{o}C for use the next day

Day 2
-Recover membranes and wash in PBS-Tween by rocking at RT for 5 min
-Do two more washes in PBS-Tween
-During this time make a 1:10,000 dilution of secondary antibody in 1% milk
-After the last wash is done, add 5-15 mL of the secondary to each of the membranes
-Rock at room temp for 45 min (set a timer, more time and you'll get non-specific bands)
-After the secondary incubation, wash membranes 3x in PBS-Tween as before
-During this time, prepare the HRP reagent
-Place clingwrap on your bench and transfer membranes the clingwrap after blotting the excess PBS-Tween dry
-Add HRP reagent to the membranes and incubate at RT for approx. 5 min
-During this time, prepare the film developer in the dark room
-Transfer membranes to an exposure cassette after blotting excess HRP reagent from them
-Take 30 s and 5 min exposures, develop the film and decide whether shorter, intermediate, or longer exposures are necessary