Casting Microwestern Gels

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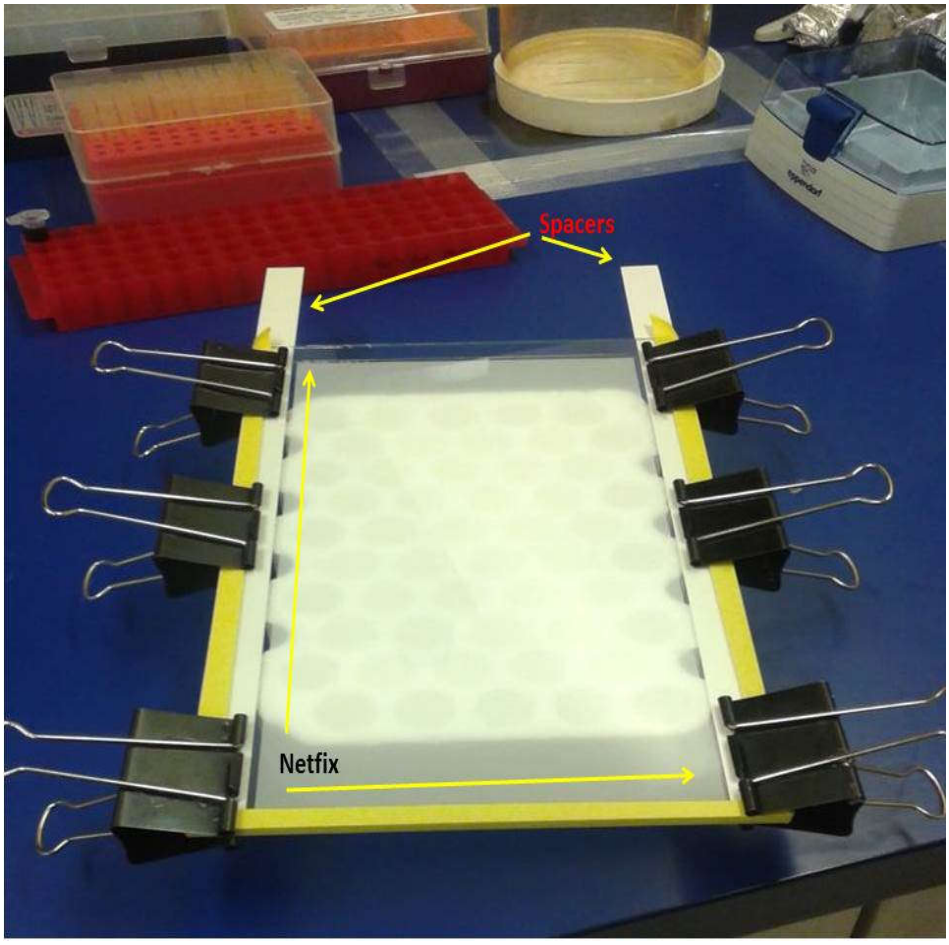
Validated by / Date:

NOTE: This protocol was inspired by that written at U. Chicago by Mark Ciaccio (Ciaccio MF, Wagner JP, Chuu CP, Lauffenburger DA, Jones RB (2010) Systems analysis of EGF receptor signaling dynamics with microwestern arrays. Nat Methods 7:148–155; Youtube videos on MWA, www.youtube.com/watch?v=0iUhoWL1IC0).

1) Set up the Gel Cast
   a) Clean long and short glass plates (Moliterno, long plate, #GBS-160-280, 16 cm x 28 cm x 2.3 mm and short plate, #GBS-160-270, 16 cm x 27 cm x 2.3 mm)
      i) Add MilliQ water (Millipore Advantage A10 system 18.2 MΩ) to one side of each plate and wipe dry with Chemwipes (Fisher, #06-666-A)
      ii) Apply 95% EtOH (Fisher, #S25309E) to the same surfaces and squeegee with a single edge razor blade (Fisher, #12640) to remove any particulate matter. Remove excess EtOH with Chemwipes.
   b) Silanize each cleaned surface
      i) Prepare a 2.5% silane stock by combining 1.25 mL dichlorodimethylsilane (Sigma-Aldrich, #40140-25ml) and 48.75 mL 100% ethanol (Fisher, #04-355-22) in a 50 mL conical tube (Fisher,#14-432-22). Store wrapped in aluminum foil at room temperature. Record the date of preparation on the tube. The solution is good for up to 6 months.
      ii) Apply approximately 0.5 mL of 2.5% silane to each cleaned side of the glass plates by spreading drops over the surface.
      iii) Spread silane evenly over the glass surface with Chemwipes until dry. Clean surface with MilliQ water and Chemwipes as before (Step *1ai*)
   c) Place 0.4 mm deep plastic spacers (30 cm x 1.5 cm x 0.4 mm), made from white high impact styrene,on the long edges (28 cm) of the long plate

d) Center Netfix rectangle, 26.5 cm x 12.5 cm (Serva, #42500.01) between spacers.
e) Place short plate with the silanized side down on top of the spacers.
f) Tape (¾" TapeLogic vinyl electrical tape,yellow-- Staples #191530) sides and bottom of the plates and place 3 clamps (Staples #831610) along each long side (6 total) equally spaced. Even spacing ensures that the gel will have uniform thickness. (See Figure 1, below.)

Figure 1.



2) Cast the Gel
    a) Make 5x Gel Buffer, pH 6.9: 1.2M Tris Acetate (Tris; BioRad #161-0719) (Acetic acid; Sigma-Aldrich #320099)
        i) Add 145.4 g Tris base (BioRad, #161-0719) to 700 mL MilliQ water. pH should be between 11.0 to 11.4. If it is not, start fresh.
        ii) Add 65 mL glacial acetic acid (AA) (Sigma-Aldrich, #320099) while monitoring pH; pH should go down to ~7.1.
        iii) Let solution stand overnight.
        iv) Add AA in 0.5 mL increments until pH reaches 6.9.
        v) Let solution stand for at least one hour at room temperature.
        vi) Repeat steps iv) and v) until pH is stable at 6.9.
        vii) Bring volume up to 1 L with MilliQ water and store at 4°C. This stock will be good for 6 months.
        viii) Do not add sodium hydroxide (NaOH) to adjust pH. The ions may affect protein migration during electrophoresis. If the pH stably becomes <6.75 then restart at Step *2ai* and add smaller increments of AA in Step *2aiv*.
    b) In a 50 mL conical tube vigorously mix the following reagents to produce a 9.5% acrylamide gel: 9.5 mL acrylamide/bis solution (BioRad, #161-0156; 30% Acrylamide/Bis Solution, 29:1); 8 mL MilliQ water; 6 mL glycerol (Sigma #G5516-500ml) and 6 mL 5x Gel Buffer.
    c) Allow bubbles to subside by letting solution stand uncovered at room temperature for about 10 minutes. Add 0.3 mL 10% SDS (Fisher #BP 2436); 0.15 mL 10% Ammonium Persulfate (APS) and 0.012 mL TEMED (BioRad #161-0800)
        i) APS (BioRad #161-0700) 10% is made as follows: dissolve 1 g ammonium persulfate (FW 228.2) in a final volume of 10 mL MilliQ water. Store covered at 4˚C (make fresh weekly).
3) Mix gently but thoroughly by inverting, and then pour into the cast from above. The cast should be held at about a 45° angle with the bench top surface. Keep excess gel solution in the 50 mL tube at room temperature to verify polymerization. Let stand 1 hour at room temperature.
4) Cutting the Gel
    a) Carefully remove clamps and tape from around the cast.
    b) Remove the top plate carefully with a gel wedge or razor blade.
    c) Remove gel from the bottom glass plate by lifting manually from the plate with gloves.
    d) Place the gel between 2 plastic sheets (3M Transparency Film, 8.5” x 11”, #AF4300). Press out bubbles between the plastic and the gel using a rubber brayer (Staples, #WYF078276230510), pressing firmly yet without excess pressure. Excess is defined as that which causes the gel to deform.
    e) Use a paper cutter (Staples, #103450) suitable to cut plastic to remove the Netfix border.

f) Divide the remaining gel into two equal sections (each must be at least 11.5 cm wide for a 96 well hybridization plate; bigger is better).
g) Store each section in an individual plastic resealable bag, removing air by hand.
h) Record the date on the bag. Gels are stable for 3 months at 4°C.