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Midi-gels: casting and electrophoresis

1. Prepare all buffers and reagents according to recipes at the end of this document. What you'll need:

   Gel components:
   30% Acrylamide/Bis Solution*
   1 M Tris pH 8.7
   0.5 M Tris pH 6.8
   10% SDS (w/v)
   10% APS (w/v)
   TEMED*

   **premade solutions available from BioRad and stored in fridge*

   Additional reagents:
   Water-saturated butanol
   Pasteur pipet
   Filter paper
   26-well midi-gel cassettes (ThermoFisher cat no. WC1026)

2. Remove plastic comb from gel cassettes and position on a flat surface - make sure that cassette cannot be easily knocked over. Gather serological pipets and 50 mL conical tubes for mixing gel solutions.

3. Combine **resolving gel** solution components, leaving out APS and TEMED. ***See Table 1 for recipe.***

4. Add 10% APS, gently mix without introducing air bubbles. Quickly add TEMED and mix again followed by immediately pouring the gel. Fill the cassette up to the plastic notches found on both sides (~2/3 from the bottom). Keep the remaining gel solution on your bench to gauge whether polymerization has occurred.

5. Add water-saturated butanol (using glass Pasteur pipet) to the top of the resolve gel. Add by dispensing on one side and allowing it to slowly spread out along the gel. Allow the gel to polymerize completely (~20-30 min).

6. While waiting, combine **stacking gel** solution components, leaving out APS and TEMED.

7. Once resolving gel is polymerized, pour off butanol from the top of the gel. Rinse away residual butanol with MilliQ water and then use small pieces of filter paper to remove excess liquid from the gel interface.

8. Place plastic comb back in the gel cassette. Do not insert completely at this step to allow space to pour in the stacking gel.

9. Quickly add APS and then TEMED to stacking gel solution, as performed for resolving gel. Pour stacking solution into the cassette, filling up to the top of the gel. Carefully insert the plastic comb completely, making sure to avoid bubbles around the teeth of the comb. Allow the gel to polymerize completely.

10. While polymerizing, there may be an apparent loss of volume on either side of the comb. Feel free to add additional solution if necessary. Again, store leftover gel solution to gauge when polymerization is complete.

11. Casted midi gels can be used immediately once polymerized or stored submerged in running buffer in the fridge (usually okay for ~1 week).

12. To prepare for gel electrophoresis, prepare lysates per usual for western blotting. The 26-well gels fit 15 µL so you will need to scale the loading calculations appropriately: 20 uL mix of (5 µL 4X SB, 1 µL 10X DTT, remainder clarified lysate and water), load 15 µL per lane.

13. Prepare the appropriate amount of 1X Tris-Glycine Running Buffer (recipe for 10X stock provided at end of document). 1 L should be plenty for a single run.

14. Remove tape from the casted midi gel and insert into the XCell4 SureLock electrophoresis gel box (https://www.thermofisher.com/order/catalog/product/WR0100?SID=srch-hj-WR0100#/WR0100?SID=srch-hj-WR0100).

15. Pour in 1X running buffer and make sure there are no leaks in the system. Carefully remove comb from the gel and load samples. Run electrophoresis at 125 V for 1 hr 45 min, or until ladder shows sufficient separation for your application.

16. Proceed with transfer as normal using TransBlot Turbo system. Use the settings appropriate for midi gels as specified in a default by BioRad.

Table 1	**Resolving gel**			**Stacking gel**
	8%	**10%**	**12%**	**4%**
Acrylamide/Bis (30%/0.8%)	8 mL	10 mL	12 mL	1.33 mL
1 M Tris pH 8.7	11.2 mL	11.2 mL	11.2 mL	0 mL
0.5 M Tris pH 6.8	0 mL	0 mL	0 mL	2.5 mL
10% SDS	300 µL	300 µL	300 µL	100 µL
10% APS	200 µL	200 µL	200 µL	60 µL
TEMED	20 µL	20 µL	20 µL	6 µL
ddH20	10.4 mL	8.4 mL	6.4 mL	6 mL
total	30 mL	30 mL	30 mL	10 mL

10% resolving gel (grey) is usually most appropriate for separation of proteins between 30-100 kDa. 4% Stacking always used. Scale down volumes appropriately if only making a 1 or 2 gels.

10X Tris-Glycine Running Buffer (1 L)
30.3 g Tris Base
144.1 g Glycine
10 g SDS
MilliQ water to 1 L

1 M Tris pH 8.7 (1 L)
121.14 g Tris Base, dissolve in water (up to but below 1L), adjust pH using HCl/NaOH, volume up to 1 L

0.5 M Tris pH 6.8 (1 L)
60.57 g Tris Base, dissolve in water (up to but below 1L), adjust pH using HCl/NaOH, volume up to 1 L

10% (w/v) SDS (100 mL)
10 g SDS in 100 mL water

10% (w/v) APS (10 mL)
1 g SDS in 10 mL water
APS needs to be prepared fresh! Alternatively, store smaller aliquots at -20°C