NuPAGE gel casting protocol

(updated 2017-04-03 by **P. Georgescu**)

Quick Guide

cast resolving gel (10%/10 ml) for 2 gels

add the following in 50 mL conical tube:

1. 3.33 ml **AA/bis (4ºC)**
2. 0.94 ml **7x BisTris Puffer (4ºC)**
3. 5.66 ml **H_2O**
4. 20 μl **TEMED (RT)**
5. 40 μl **APS (4ºC)**

Mix by inverting the tube.

overlay every single gel by with **500 ml isopropanol (RT, hood)**

let **acrylamide polymerize for at least 2 hours**.

drain isopropanol and rinse **thoroughly several times** with deionized water

cast stacking gel (4%/10 ml)

mix the following in a 50 mL conical tube:

1. 1.32 ml **AA/bis (4ºC)**
2. 0.94 ml **7x BisTris Puffer (4ºC)**
3. 7.68 ml **H_2O**
4. 20 μl **TEMED (RT) and store at RT** until ready to be poured. Before pouring add
5. 40 μl **APS (4ºC)** and mix by inverting the tube.

pour 2-3 ml onto each gel

insert combs

let sit for 10-15 min, then cover with a wet paper towel and parafilm

let completely **polymerize** overnight at 4 deg (fridge)

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NuPAGE gel electrophoresis protocol

(updated 2017-04-03 by **P. Georgescu**)

cleaning and assembling glass plates

1. clean short and spacer glass plates with mild detergent
2. rinse plates with deionized water and let them dry on a plate stand
3. clean plates with 100% ethanol using kimwipes
4. put one separating sheet on the back of the casting chamber
5. assemble then all plates with **spacer plate** – **short plate** (make sure the bottom of each assembly is smooth)
6. put gray sponge on the bottom of the Biorad halter and position assembly with the short plate facing out (tip: stick microtube between the upper clamps to press the assembly further into the sponge)

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1. **pour gel mix** directly into Biorad single casting chamber, fill to upper edge of the clamp
2. **overlay** every single gel by with **500 ml isopropanol** by pipetting between the gaps
3. let the **acrylamide polymerize for at least 2 hours**.
4. drain the isopropanol and rinse **thoroughly several times** with deionized water
5. dry the gel/glass tacks by letting sit upside down on paper towels for 5-10 min

casting stacking gel (4%) for 2 gels

mix the following in a 50 mL conical tube:

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3. 7.68 ml **H_2O**
4. 20 μl **TEMED (RT) and store at RT** until ready to be poured. Before pouring add
5. 40 μl **APS (4ºC)** and mix by inverting the tube.

1. **pour gel mix** onto each separating gel in between the gaps between the gel/glass plate stacks (each gel -> approx. 2-3 ml)
2. **insert combs** by pushing them softly in a 45 deg angle.
3. let the **acrylamide polymerize for at least 3 hour** (or overnight at 4 deg; cover gels with a wet paper towel and aluminum foil or parafilm when stored o/n)

4. **disassemble** carefully the gel stacks by lifting up the front lid, then taking out the entire gel stacks and disassemble them one by another. Remaining polyacrylamide traces on the top and the edges of the glass plates are removed by rinsing them with H20.
5. **storage**: enwrap every single gel with slightly wet paper towel (excess of water will be soaked up by the gel and result in changed buffer conditions) and store them together in a closable plastic bag at 4 deg.

running

- fill both chambers with either 1x MOPS-Tris or 1x MES-Tris buffer
- add 0.5 ml of 200x reducing agent (1 M sodium bisulfite) to the inner (cathode) chamber (chamber volume is approximately 100 ml).
- run at 7W constant for 1 gel or 13W for 2 gels.

note: bromophenol commonly used in loading buffer runs around 3-5 kDa with the MES-Tris buffer system.

How to order & prepare buffer and solutions:

1. **AA/bis** (Acrylamide/Bis-acrylamide 37.5:1; 30%; stored **at 4°C**)
2. **7x BisTris Buffer** = 2.5 M BisTris, 1.5 M HCl (200 ml, stored at **4°C**)
   - dissolve 104.62 g BisTris (MW = 209.24 g/mol) in 160 ml H_2O
   - add 20 ml concentrated HCl (37% or 12.08 M)
   - fine adjust pH to pH 6.5-6.8 by adding dropwise more conc. HCl
   - fill up to 200 ml with H_2O
3. **TEMED** (stored at RT)
4. **10% APS** (ammonium persulfate)
   dissolve 10 g APS (stored at RT in cabinet) in 100 ml H20
   store in 500 ul aliquots **at -20°C, working stock at 4°C**
5. **Millipore water** (does not need to be autoclaved)
6. **isopropanol (**stored **at RT**, cabinet)

for gel running:

- **20x MOPS buffer** for separating proteins **> 20 kDa**
  - 1 M MOPS (MW = 209.26 g/mol)
  - 1 M TrisBase (MW = 121.14 g/mol)
  - 2% SDS (10% stock)
  - 20 mM EDTA (MW = 372.24 g/mol, 0.5 M stock)
  - no pH adjustment necessary
- **20x MES buffer** for separating small proteins **2-50 kDa**
  - 1 M MES (MW = 195.20 g/mol)
  - 1 M TrisBase (MW = 121.14 g/mol)
  - 2% SDS (10% stock)
  - 20 mM EDTA (0.5M stock)
  - no pH adjustment necessary
- 200x reducing agent: 1 M sodium bisulfite (MW = 190.1 g/mol) added to running buffer in inner chamber (cathode) at 5 mM final concentration; be cautious, bisulfite is extremely stinky!