Simplified instructions for Invitrogen NuPAGE Gels

Running NuPAGE Gels

1. Select the desired Running Buffer (MOPS works for >200 to 14 kDa and MES for 60 to 2.5 kDa) and make up 800 ml using the 20X stocks stored at 4 degrees.

2. Remove precast gel from bag, rinse with water. Peel off tape on back of gel and remove comb. Wash out wells a total of three times with 1X running buffer using a pasteur pipette. Fill the sample wells with buffer and remove all air bubbles.

3. Set up mini cell apparatus as per instructions.

4. Fill the upper buffer chamber with about 200 ml running buffer and check for leaks. If OK, add the remainder of buffer to the lower buffer chamber.

5. For reducing SDS PAGE, add 0.5 ml antioxidant to the 200 ml of running buffer in the upper chamber. For optimum results add within 15 min of actually running the gel. This helps keep the samples reduced as the gel runs (the DTT in the sample does not migrate in the neutral pH gels as it does in Tris-Glycine gels). *Note: almost no one in the lab uses antioxidant and this has no apparent effect.*

6. For MOPS buffer, run at 200V constant voltage for ~50 min. Expect 100-115 mA current at the beginning and 60-70 mA at the end.

7. For MES buffer, run at 200 V constant voltage for ~35 min. Expect 110-125 mA current at the beginning and 70-80 mA at the end.

Note: Prestained protein standards migrate abnormally in NuPAGE gels. Use See-Blue+2 pre stained marker proteins and use the Invitrogen callibration chart to determine the molecular weight of your sample.

SDS PAGE sample preparation

1. To protein sample, add 1/4 final volume 4X NuPAGE sample buffer (this is very viscous and needs to be heated to at least room temp). Add 1/10 volume reducing agent (stabilized DTT) or use your own fresh 0.5 mM DTT. Heat 10 min at 70 degrees (boiling for 2-3 min probably works as well).

2. Load samples to NuPAGE gel and run as outlined above.

The gels are 1.0 mM thick and the 15 well comb can hold a total of 15 microliters and the 12 well comb can hold a total of 20 microliters.

Electroblotting

(blotting two gels in the same transfer cassette doesn't work well)

1. Prepare 400 ml NuPAGE transfer buffer (using 20x transfer buffer stock - kept at 4 deg)

final concentrations in transfer buffer:

1.25X Transfer Buffer

5% Methanol

Final volume 400 ml

Note: depending on the protein properties, can use up to 15% Methanol. Increased methanol concentration will aid in protein binding to the membrane, but may inhibit the protein elution from the gel. In general, use the lower methanol concentration if your protein is large or has a problem transfering out of the gel. We have found that 5% Methanol works well for many proteins.

0.4 ml antioxidant (100x) (not necessary unless you will be sequencing the protein or trying to recover activity later)

2. Use 0.45 micron PVDF membrane (immobilon-FL has low fluorescence and is used with the Odyssey system). Prepare PVDF membrane by pre wetting for 30 seconds in methanol, rinse briefly in water, and then soak in ~20 ml transfer buffer for >10 minutes (do not presoak the gel).

3. Presoak the blotting pads in transfer buffer (no air bubbles). Transfer works best if these are presoaked for >20 min.

4. After opening the gel cassette, remove the wells with a knife. Place a piece of pre wetted filter paper on the gel, leaving the foot uncovered (make sure there are no trapped air bubbles using a pipette).

5. Turn the cassette over and cut the foot off the gel using a knife.

6. Wet the surface of the gel with transfer buffer and position the presoaked PVDF membrane on the gel (no air bubbles). Place a piece of presoaked filter paper on top of the PVDF membrane.

7. Assemble the sandwich in the transfer apparatus as shown in the NuPAGE instructions. Fill the chamber with ~200 ml transfer buffer until the gel + membrane is covered.

8. Fill the outer buffer chamber with ~600 ml deionized water (necessary to dissipate heat during the transfer).

9. Run the unit at 30 V constant voltage for 1 hr. If you are in a hurry, can run at 36 V, 45 min. For large proteins which have a problem eluting from the gel, the transfer can be run for as much as 2.5 hr. Be aware that longer transfer times may cause smaller proteins to pass through the membrane.

NuPAGE Buffer Recipes

(20 X) MOPS/SDS running buffer (1 liter)

MOPS	209.2 g	1.0M
Tris base	121.2 g	1.0M
SDS	20 g	69.3 mM
EDTA free acid	6.0 g	20.5 mM

Ultrapure water to 1.0 liter final concentration. 1X buffer should be pH 7.7 (do not adjust with acid or base).

(20 X) MES/SDS running buffer (1 liter)

MES	195.2 g	1.0 M
Tris base	121.2 g	1.0 M
SDS	20 g	69.3 mM
EDTA free acid	6.0g	20.5 mM

Ultrapure water to 1.0 liter final concentration. 1X buffer should be pH 7.3 (do not adjust with acid or base).

(20X) NuPAGE transfer buffer (500 ml)

Bicine	40.8 g	(500 mM)
Bis-Tris	52.32 g	(500 mM)
EDTA free acid	3.0 g	(20.5 mM)

Ultrapure water to 500 ml. 1X buffer should be pH 7.2 (do not adjust with acid or base).

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