

NON-DENATURING PAGE GEL

Gel rig = holds 5 gels

Two white metal and three glass plates

1st whole plate, then spacers, then rabbit ear plate

Hold together with 4 clips

Rig holds 50 ml

12% GEL

20 ml 30% acrylamide/bisacrylamide (19:1)

10 ml 5X TBE

20 ml ddH₂O

600 ul AP (10% ammonium persulfate – 0.2g in 2 ml)

10 ul TEMED

Fill with 10 ml pipette to top

Add combs

Place remaining liquid into 15 ml to watch timing of polymerization

When gels are polymerized

Remove clips

Separate with razor blade

Run under H₂O

Cut away excess gel

Carefully remove combs

Wrap in Saran

RUNNING PAGE GELS

Place gel in chamber with two clips, rabbit ears to back

Add 1X TBE to gel (fill to top of glass)

Add TBE to bottom of rig (both sides)

Add 1 (3-4 ul) part to 4 parts, TBE sample buffer to 10-12 ul of sample

Add sample to wells

Run at 140V constant voltage (=10-13 milli amps)

Run until dark violet line reaches bottom of gel

Remove plate, remove spacers, separate plates with razor

Run H₂O over gel to loosen from plate

Knotch top left with razor to maintain orientation

Stain in 1 ug/ml ethidium bromide – shaking 10 min

Recycle stain