NEXAGENLABS · BENCHMATE

Gel Loading Dyes

Tracking dye migration by gel type and percentage, plus the standard DNA and SDS-PAGE loading buffer recipes.

Dye migration (apparent DNA fragment size)

Gel	Bromophenol blue	Xylene cyanol FF
Agarose, 1%	~300–500 bp	~3,000 bp
Acrylamide (non-denaturing), 3.5%	~100 bp	~460 bp
Acrylamide (non-denaturing), 5.0%	~65 bp	~260 bp
Acrylamide (non-denaturing), 8.0%	~45 bp	~160 bp
Acrylamide (non-denaturing), 12.0%	~20 bp	~70 bp
Acrylamide (non-denaturing), 15.0%	~15 bp	~60 bp
Acrylamide (non-denaturing), 20.0%	~12 bp	~45 bp

Orange G runs faster still, ~50 bp in 1% agarose. Run a sized ladder alongside the dye front for anything other than a rough visual check.

6× DNA loading buffer

Component	Glycerol-based	Ficoll-based
Density agent	30% (v/v) glycerol	15% (w/v) Ficoll 400
Bromophenol blue	0.25% (w/v)	0.25% (w/v)
Xylene cyanol FF	0.25% (w/v)	0.25% (w/v)

Ficoll 400 avoids glycerol's band-wobble in slab gels and does not need the sample kept cold, at the cost of being slower to dissolve.

4× Laemmli SDS-PAGE sample buffer

Component	4× amount
Tris-HCl, pH 6.8	250 mM
SDS	8% (w/v)
Glycerol	40% (v/v)
Bromophenol blue	0.02% (w/v)
Reducing agent (add fresh)	DTT to 400 mM, or 2-mercaptoethanol to 8% (v/v)

Add the reducing agent fresh: DTT and 2-mercaptoethanol both oxidise over hours to days once diluted.

Source: dye migration and DNA loading buffer recipes from Sambrook & Russell, Molecular Cloning: A Laboratory Manual, 3rd edition, Appendix, corroborated against Bio-Rad's public dye-migration reference. Laemmli sample buffer from Laemmli (1970), Nature 227:680-685, checked against Bio-Rad's published Laemmli buffer literature.

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For the theory behind gel electrophoresis, see the Biotech Techniques Bible series.