Electrophoresis loading buffers.

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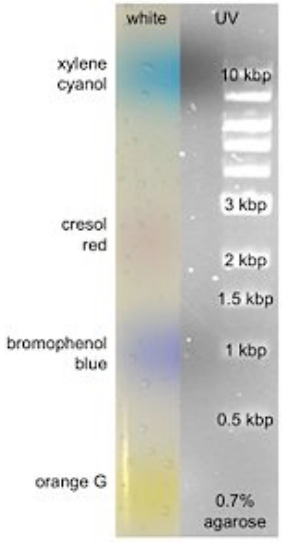
This protocol provides detail into the preparation of electrophoresis loading buffers for use in agarose or acrylamide gel electrophoresis of nucleic acids. The PCR product and genomic DNA or total RNA (if nucleic acid integrity is to be evaluated) are loaded into gel wells after mixing with 6x loading buffers which minimize well-to-well carryover or spill and allow for visualization of electrophoretic progress thanks to several dyes.

6x Xylene Cyanol loading buffer (nominal migration 4 to 10 kbps)

In 1% agarose gels, xylene cyanol migrates at about the same rate as a 4 to 5 kilobase pair DNA fragment, although this depends on the buffer used. Xylene cyanol on a 6% polyacrylamide gel migrates at the speed of a 140 bp DNA fragment. On 20% denaturing (7 M urea) polyacrylamide gel electrophoresis (PAGE), xylene cyanol migrates at about the rate of 25 bp oligonucleotide.



- 25 mg of Xylene Cyanol
- 3 mL of Glycerol or 1.5 grs of Ficoll-400 or 4 grs of sucrose
- Top up to 10 mL with dH_20.

6x Bromophenol Blue Loading Buffer (nominal migration 300 bp)

Bromophenol blue (3′,3″,5′,5″-tetrabromophenolsulfonphthalein, BPB, albutest) is used as a pH indicator, an electrophoretic colour marker, and a dye. As an acid–base indicator, its useful range lies between pH 3.0 and 4.6. It changes from yellow at pH 3.0 to blue at pH 4.6; this reaction is reversible. Bromophenol blue is structurally related to phenolphthalein. In a typical 1% agarose gel in a 1X TAE buffer or TBE buffer, bromophenol blue migrates at the same rate as a DNA fragment of about 300 base pairs, in 2% agarose as 150 bp.

- 25 mg of Bromophenol Blue
- 3 mL of Glycerol or 1.5 grs of Ficoll-400 or 4 grs of sucrose
- Top up to 10 mL with dH_20.

NOTE: Commercial (Sigma-Aldrich G2526) Agarose Gel loading buffers contain 0.05% bromophenol blue, 40% sucrose, 0.1M EDTA (pH 8.0) and 0.5% SDS.

6x Orange G Loading buffer (nominal migration 50 bp)

Orange G also called C.I. 16230, Acid Orange 10, or orange gelb is a synthetic azo dye used in histology in many staining formulations. It usually comes as a disodium salt. Orange G is used in the Papanicolaou stain to stain keratin. It is also a major component of the Alexander test for pollen staining. It is often combined with other yellow dyes and used to stain erythrocytes in the trichrome methods. It exhibits two colours in aqueous solution, brilliant orange in neutral and acidic pH or red in pH greater than 9.

- Mix 18 mL of Glycerol with 40 mL of dH_20.
- Add 60 mg of Orange G dye and top up to 50 mL.
- Vortex.

6x Mixed Loading Buffer (Bromophenol blue and Xylene Cyanol)

This loading buffer has been amply used in commercial preparations as it provides two dye migration bands on agarose electrophoresis which provide a better visualization of the estimated PCR product migration. However, both of these dyes can obscure PCR bands of similar migration size making test result interpretation difficult.

- 25 mg of Bromophenol blue
- 25 mg of Xylene Cyanol
- 3 mL of Glycerol or 1.5 grs of Ficoll-400 or 4 grs of sucrose
- Top up to 10 mL with dH_20.

Cresol Red Loading Buffer (nominal migration of 125 bp)

Cresol Red does not inhibit Taq polymerase to the same degree as other common loading dyes as such it can be incorporated into PCR for immediate loading unto gel after cycling. Provides much less shadow on EtBr stained gels unlike xylene cyanol and bromophenol blue. Can replace entire volume of dH_20 used in PCR for 1% Cresol Red dye solution. When prepared with Glycerol the final concentration of this Cresol Red Loading buffer in the PCR must not be greater than 2%.

- Add 500 mg of Cresol Red dye to 50 mL dH_20 to prepare a 1% solution. Vortex
- Add 17 g of sucrose to 49 ml of dH_20 in a 50 ml Falcon tube. Vortex. Alternatively,
- Add 15 mL of Glycerol to 49 ml of dH_20 in a 50 ml Falcon tube. Vortex
- Add 1 ml of 1% Cresol Red dye solution. Vortex

6x UC Berkeley Loading Dye

Ficoll®-400 results in brighter and tighter bands when compared to glycerol loading dyes. SDS creates sharper bands, as some restriction enzymes are known to remain bound to DNA following cleavage. EDTA chelates magnesium (up to 10 mM) in enzymatic reactions, thereby stopping the reaction. Tris-HCl buffers the sample at a pH safe for DNA. This 6× loading dye recipe is identical to that of NEB loading dyes, except for the addition of both xylene cyanol and Orange G (slightly reduced from 0.15%) with bromophenol blue. The Dueber Lab also adds 6× GelGreen DNA stain to the loading dye, so as not to have to pre/post-stain the gel.

- 1.5 g Ficoll-400 for a 10 mL final buffer volume.
- 3.33 mM Tris-HCL, pH 8.0 final concentration.
- 10 mM EDTA final concentration.
- 0.08% SDS final concentration.
- 3 mg Xylene Cyanol for a 10 mL final buffer volume.
- 3 mg Bromophenol Blue for a 10 mL final buffer volume.
- 12 mg Orange G for a 10 mL final buffer volume.

Dye	Mobility in 1% agarose	Mobility in 2% agarose
Xylene cyanol	4 kb to 5 kb	200 to 750 bp
Cresol red	≈ 1 kb to 2 kb	≈ 100 bp
Bromophenol blue	400 to 500 bp	50 to 150 bp
Orange G	< 100 bp	?
Tartrazine	< 20 bp	< 20 bp

Notes

1. Store buffers prepared with ficoll or sucrose in a refrigerator between 0ºC to 4ºC to avoid contamination.

Revision history

1.0 Original document.

2.0 Changes to document format only.

3.0 Translated to English.