SGTB Agarose Electrophoresis Buffer

 # GB01.0510 (5L 10X) | #GB01.0120 (1L 20X) | #GB01.0520 (5L 20X)

(FOR RESEARCH ONLY)

Product Description

SGTB is a new electrophoresis buffer ideal for agarose gel electrophoresis of small DNA fragments (up to 1000bp). Unlike gels prepared with TAE or TBE, which tolerate only about 110V (mini-gel), the low-conductance electrophoretic buffer SGTB allows for the application of high voltage (200V-300V for a mini-gel) without generating a lot of heat, and without melting your gel. This allows for much faster runs. Not only does this save time, but faster runs result in less fuzzy bands, and therefore, using SGTB results in very sharp bands. Moreover, in comparison with TBE and TAE, using SGTB results in larger differences in relative mobility (Rf), especially between smaller DNA fragments, allowing for a better separation of DNA bands of similar size. On the other hand, if you already get the resolution you need, with SGTB you can achieve the same result using less agarose, thus not only saving you time but money as well. Finally, gels prepared with SGTB are clearer than gels prepared with TAE, increasing detection sensitivity, and also stronger, making them easier to handle.

Preparation

SGTB is prepared with ultrapure water and 0.2µm filtered, and is provided as a concentrated aqueous solution (either 10X or 20X concentrated). The working concentration is 1X. Prepare 1L SGTB Buffer (1X) by mixing 100ml of a 10x concentrated buffer with 900ml of ddH_2O, or alternatively, 50ml of a 20x concentrate with 950ml of ddH_2O. SGTB (1X) should be used both for the preparation of the agarose gel as well as the running buffer. Like TBE (and unlike TAE), SGTB does not exhaust quickly during extended electrophoresis, and so the buffer may be used multiple times.

Storage

SGTB Buffer (10X or 20X) should be stored at room temperature and is stable for at least one and a half years.

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Usage

Prepare 1X SGTB DNA Electrophoresis Buffer by diluting the concentrate in pure water. Prepare the agarose gel with 1x SGTB as normal (like with TAE or TBE). Fill the electrophoresis chambers with 1x SGTB and put the gel casting tray into place. It is recommended to wash the electrophoresis chambers the first time with SGTB to remove any residual TAE or TBE (if applicable). For optimal resolution, cover the gel with no more than 3-5mm of SGTB. Apply samples and run the gel at constant voltage, using a higher voltage than normal. GRiSP suggests to start by doubling the normal voltage one normally uses for either TAE or TBE and then gradually increase voltage to find the optimum. The optimal voltage depends, among others, on the type and percentage of agarose, as well as on the nature of the sample. Therefore, occasionally the optimal voltage might be less than 2x the normal voltage. Too high voltage does not result in melting of the gel, however, DNA bands might become distorted. The run time will be reduced considerably and will be roughly reduced by the same value as the increase of the voltage. For example, when normally running a gel for at 100V for 1 hour, start with running at 200V for 30 minutes. Then, determine whether the electrophoretic conditions might be further improved (voltage, time, percentage agarose). It is strongly advised to determine optimum run time empirically, and not to rely on the tracking dye, as the behavior of tracking dyes depends highly on the electrophoresis buffer and the percentage of agarose (see section about tracking dyes hereunder).

Tracking Dyes

Nucleic acid agarose gel electrophoresis loading buffers contain one or more tracking dyes in order to help with pipetting and to be able to visually follow the electrophoresis progress. Common dyes are Bromophenol blue (BPB), Xylene Cyanol FF (XC), Orange G (OG), and Cresol Red (CR). Migration of these dyes depends, among other factors, on the electrophoresis buffer used. In general, when using GRiSP´s new electrophoresis buffer SGTB, tracking dyes migrate much faster than with either TAE or TBE, that is: co-migrate with smaller DNA fragments than usual. The following table compares the approximate migration rates of tracking dyes using various concentrations of a standard agarose in TAE, TBE, and SGTB. Note that other factors, such as the type of agarose and the DNA sample, are also of influence and that, therefore, these values should only be used as a rough estimation.

% agarose	Xylene Cyanol FF			Cresol Red	Bromophenol Blue			Orange G
	SGTB	**TAE**	**TBE**	**SGTB**	**SGTB**	**TAE**	**TBE**	**SGTB**
0.25/0.30*	> 25 kb	25 kb	19 kb	2,5-3,0 kb	1,5 kb	2,9 kb	2,9 kb	400 bp
0.50	15 kb	11 kb	12 kb	750 bp	400 bp	1,7 kb	1,4 kb	60 bp
0.75	12 kb	10 kb	9 kb	325 bp	200 bp	1,0 kb	700 bp	50 bp
1.00	10 kb	6 kb	4 kb	250 bp	125 bp	500 bp	400 bp	<40 bp
1.25	2,1 kb	3,6 kb	2,5 kb	170 bp	90 bp	370 bp	250 bp	<40 bp
1.50	900 bp	2,8 kb	1,8 kb	100 bp	50 bp	300 bp	200 bp	< 40 bp
1.75	n.d.	1,8 kb	1,1 kb	n.d.	n.d.	200 bp	100 bp	n.d.
2.00	350 bp	1,3 kb	850 bp	50 bp	<40 bp	150 bp	70 bp	< 40 bp
2.50	250 bp	n.d.	n.d.	< 40 bp	< 40 bp	n.d.	n.d.	< 40 bp
3.00	150 bp	n.d.	n.d.	< 40 bp	< 40 bp	n.d.	n.d.	< 40 bp

*) for TAE/TBE: 0.30% and for SGTB: 0.25%; Note that bp sizes were rounded off. Data for TAE and TBE were compiled from data provided by the agarose manufacturer and thirds, and were partly confirmed by GRiSP.