



AGAROSE RPA-2500/RPS-2100

A molecular biology grade Agarose that is free of detectable nuclease activities (DNase or RNase) and suitable for analytical and preparative electrophoresis of nucleic acids. Most common uses are analysis of PCR products, plasmid DNAs, restriction enzyme digests and mRNAs or in vitro transcription products.

Hints for preparation of an Agarose gel with Agarose RPA-2500/RPS-2100:

Standard method of preparation of a gel is to use either TBE (Tris-Borate-EDTA) buffer or TAE (Tris-Acetate-EDTA) buffer. Weight out the desired amount of Agarose RPA-2500/RPS-2100 in a suitable volume of buffer (for example, 1gm per 100ml buffer for a 1% gel). Place the mixture in a container that is at least five times the volume of the Agarose solution. Place in microwave and heat in multiple short pulses (20-30 seconds) with stirring in between until all Agarose is melted into a uniform free flowing clear solution. Let it cool to warm (about 45- 50C) while stirring. Add 1-2 ul of a 10mg/ml Ethidium Bromide solution or a less toxic alternative like Gel Red from Phenix Research and stir to mix. Stir by swirling in order to avoid bubbles being trapped in the viscous liquid. Pour in gel chamber, place comb and allow solidifying for 30-45 min, placing gel in buffer chamber, loading samples and running at desired settings (constant voltage or amp).

General Properties:

Agarose RPA-2500/RPS-2100 is suitable for use in gels as low as 0.7% to as high as 2.2%. In a typical experiment, a 2% gel will resolve PCR products or restriction digest fragments as small as 80bp while a 0.7% gel will resolve up to 12 Kbp.

Excessive Heating to Dissolve Agarose:

Different preparations of Agarose used in molecular biology differ in how they dissolve. It is always better to avoid boiling (bubbling over) and the best way to avoid is to use multiple short pulses of microwave. Overheating and boiling over can compromise the outcome of analysis in unpredictable ways.

Staining for Nucleic Acid Visualization:

Customarily, ethidium bromide staining is effective in most standard analytical processes. Incorporating the ethidium bromide in the gel itself is convenient provided one is not trying to distinguish between closed super coiled and nicked circular plasmid DNAs since ethidium bromide binding relaxes super coiled DNAs and affect their mobility. An alternative is to stain the gel after the run is complete with one of many staining dyes. For high sensitivity staining dyes such as Sybr Green, it is recommended that the gel be pre-



electrophoresed before running DNA samples. This frequently removes potential interfering materials in the buffer or the Agarose. It is also important to remember that running a gel at a high voltage that leads to heating of the buffer (which sometimes allows better resolution) can soften the gel. This could change the gel's handling after the run and also affect quality of staining.

Pre-electrophoresis of Agarose Gels:

Many recommend running the gel for 15-30 minutes at the settings to be used for the actual run. Most often this is not necessary. In the case of mini-gel chambers that use small volumes of the buffer, it is recommended that you replace the running buffer with fresh running buffer before the actual run.

Reusing Running Buffer:

In standard gel chambers that use reasonably large volumes of running buffer, one can reuse the buffer to run a second gel. However, we recommend not doing so. In the event you decide to reuse the buffer, please make sure that the buffers from the two chambers are fully mixed and that the buffer volume to gel volume ratio is high (at least 20 times). Also, if the gel is run at a high voltage or if the intent is to resolve small DNA molecules, then it is better not to reuse the buffer.

Appearance of Agarose:

Agarose for molecular biology quite often has a granular appearance. In contrast, Agarose RPA-2500/RPS-2100 is amorphous due to the manufacturing method used. Qualitatively, this does not affect the performance of the gel. Amorphous Agarose may appear to boil more easily than granular Agarose while the latter dissolves more slowly.

Agarose should be stored in a cool, dry place. Avoid leaving an unsealed bottle in a humid/hot location or close to a place where there is significant microbial load. After all, Agarose can be perfect scaffolding for the growth of bacteria and fungus that will affect the intrinsic property of the material and affect the performance of the gels.

If you notice significant clumps or caking when you first open a bottle of Agarose RPA-2500/RPS-2100, contact your sales representative to help you exchange the bottle.

Contact your PHENIX account team members at **800.767.0665** or e-mail them at sales@phenixresearch.com.