Q & A continued...

Q. Will a Synergel/Agarose gel dissolve in sodium iodide for researchers who want to release DNA to bind with glass beads?
A. Yes.

Q. Can Synergel be used for protein electrophoresis?
A. It is not recommended.

Q. Would Synergel interfere with the random prime reaction?
A. It should not, however, we have not done the experiment. There is no problem with DNA ligation, kinasing and restriction fragment digestion.

Q. How do I prevent gel well tearing after the Synergel/ Agarose gel is formed prior to electrophoresis?
A. The comb may have been allowed to remain in the solidified gel too long after the gel had set. Use the buffer to keep gels moist after setting to facilitate removal of combs. Always remember to use clean combs.

Q. Will products such as agarase or "Gelase" from Epicenter, Inc. allow one to recover nucleic acids?
A. No, they are enzymes that will digest agarose but not Synergel. Thus one would still have to fractionate DNA from the Synergel. It is therefore recommended to use either a glass binding recovery system, e.g. GeneCleanX, electroelution, blotting or elution.

Q. What is the minimum amount of agarose needed to ensure gel strength with Synergel?
A. We recommend 0.7% agarose.

Q. For electrophoresis, Synergel is working fine, but why is it not blotting for some nucleic acids, such as large mRNAs?
A. Partial alkaline hydrolysis may be required to improve the transfer of high molecular weight RNA. Soak the gel in 50mM NaOH and 10mM NaCl for 45 minutes at room temperature. This will cause partial hydrolysis. Now neutralize the gel in 1.0M Tris-HCl for 30 minutes. Then soak the gel in 10-20X SSC buffer and transfer by capillary blotting per the Maniatis protocol.

Q. Will the presence of Synergel effect the ability of ethidium bromide to bind to DNA?
A. No.

Q. Why won't genomic DNA transfer as well in a Synergel/ Agarose gel using capillary blotting as it would with agarose alone?
A. Although the Synergel/Agarose mix forms a binary gel structure, traces of Synergel may compete with DNA for binding to the filter during capillary blotting, thereby reducing the DNA transferability. Electroblotting is recommended.

Ordering Information

Cat. No.	Size
SYN-100	100g

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Gel Drying Frames - Easily dry gels without the use of expensive heat/vacuum gel dryers. Use our 24cm x 24cm frames for large gels and our 14cm x 14cm frames for smaller gels.

Gel Handler - Flexible UV transparent sheets for handling gels, visualizing and photo documentation. Gel Handler can be autoclaved and acid-washed without a chemical reaction and will protect the transilluminator surface from cuts and scratches.

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SYNERGEL™

Improving Agarose Gel Performance

- **Superior Sieving Ability** - 1% Synergel is the functional equivalent of 2% agarose and 2% Synergel is the functional equivalent of 4% agarose.

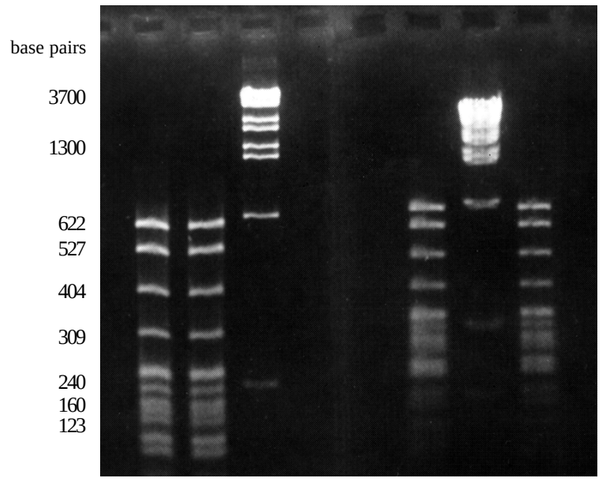
- **High Resolution** - Gels exhibit improved spacial separation and tighter banding, particularly for DNA fragments of 50-2000bp.

- **Clearer Gels with no background fluorescence** - The superior optical clarity of Synergel permits high quality photodocumentation.

- **Increased loading capacity** - Synergel can accomodate greater quantities of DNA, thereby reducing band tailing and smearing.

- **Economical** - Use of Synergel greatly reduces gel costs.

- **Easy to use**- Mix the Synergel powder with agarose, boil and pour. Gels set more rapidly than those made with agarose alone.



A. 1.5% Synergel and 0.7% agarose (SeaKem® LE)[1]
B. 4% NuSieve® agarose[2]

[1]SeaKem® is a registered trademark of the FMC.
[2]NuSieve® is a registered trademark of Biowhittaker Molecular Applications (BMA).

The Synergel Technology

Synergel is a synergistic gelling and sieving agent consisting of a modified polysaccharide which, when combined with agarose, forms a hydrogen bonded binary gel system. This type of gel is easily constituted by weighing out the Synergel and agarose powders, dissolving them by boiling in buffer and pouring the gel. The addition of Synergel to agarose improves gel performance by providing improved separation and definition of DNA fragments. A gel mixture containing 0.7% agarose and 1.0% Synergel will produce improved results when compared to a 2% agarose-only gel. Separation of small DNA fragments in 2% Synergel gels is comparable to, or better than that observed in 4% NuSieve®[2] agarose gels. Synergel is chemically modified to allow a greater concentration of the polymer to be incorporated into the gel structure, thereby enhancing molecular sieving in the gel. Results show improved spatial separation and resolution of DNA fragments in Synergel containing gels as compared to agarose-only gels. Good spatial separation of the fragments and tight banding patterns are evident for DNA fragments of 50-2000bp in size. Gels containing lower concentrations of Synergel (0.5%) plus agarose were found to be very useful for separating larger DNA fragments (1-30bp). Synergel also provides greater optical clarity, which allows higher quality photodocumentation of stained gels.

The Synergel/Agarose gel sets at approximately the same temperature, but somewhat more rapidly than agarose alone. Phosphate, acetate or borate buffered Tris-EDTA may be used in forming the gel. Borate buffers may also be used to form the gel, but are slightly more viscous and therefore should be poured at a slightly warmer temperature. Compared to simple agarose, the Synergel/Agarose gel is stronger and more transparent. Synergel is DNase and RNase free. DNA bands may be extracted and recovered using glass suspension systems, e.g. Geneclean™. DNA may also be electroeluted or transferred to membranes following standard Southern blotting protocols. RNA may also be fractionalized in this system by using a 2.2M formaldehyde-containing buffer.

Step-by-Step Instructions:

Materials Required:

Synergel
Agarose
Ethanol
Buffer: 0.5X TPE Buffer (0.5X) solution: 0.04 Tris base, 0.004M EDTA, pH adjusted to 8.0 with phosphoric acid. Alternatively, 1X TAE or 0.5X TBE may be used.

Instructions:

1. Weigh out the required amount of agarose and place in a dry beaker or flask. Agarose concentration is generally fixed at 0.7% (w/v).

2. Calculate and weigh out the appropriate amount of Synergel, which is generally between the range of 0.2-2.0% (w/v). Higher concentrations of Synergel (3-4%) may be used for fractionating very small molecules (100bp or less). The Synergel concentration is derived by taking the normally used agarose concentration, subtracting 0.7% and multiplying by 0.5.

3. Add the Synergel powder to the beaker or flask and thoroughly intermix with the agarose.

4. Thoroughly saturate the mixed powders with ethanol. (Enough ethanol so that the resulting slurry can move about freely at the bottom of the container.)

5. Gradually add 0.5X TPE buffer or alternative buffer while swirling the container to prevent clumping.

6. Dissolve the suspension in a microwave or by direct heating. Intermittent swirling during the heating process helps to ensure the best dispersal, avoids boil over and facilitates rapid dissolving of the suspension. To be sure that all particles are dissolved, hold the suspension to the light, if no refractile particles are visible, the solution may be cooled and poured. If particles persist, re-microwave to boiling then let solution sit for approximately 30 seconds. Repeating this procedure 2-3 times may be required before particles dissolve into solution. Gelling temperature will be approximately the same as set for the agarose component alone. After the gel has "set" remove the comb and add buffer in preparation for electrophoresis.

Troubleshooting Note:

The use of very clean combs facilitates easy removal of the combs and prevents tearing of the gel walls.

Reference:

Synergel represents a second generation modified polysaccharide product related to the gel composition described in *Analytical Biochemistry* 163:247-254 (1987).

How to Calculate Required Synergel

Easy, step-by-step instructions to calculate the amount of Synergel needed to add to agarose when making the functional equivalent Synergel/Agarose gel.

1. What percentage agarose gel is currently being used?
2. Subtract 0.7% from the agarose total.*
3. Divide the difference by 2. The result is the percentage of Synergel to be added to the 0.7% agarose.

*The 0.7% is the minimum agarose concentration required to ensure gel stability.

Examples:

Convert a 1% agarose to a 1% Synergel/Agarose gel

a. Subtract 0.7% from the 1% agarose; 1 - 0.7 = 0.3%
b. Divide the difference by 2; 0.3 ÷ 2 = 0.15%
c. 0.15% is the amount of Synergel added to 0.7% agarose to produce the functional equivalent of a 1% agarose gel.

Convert a 3% agarose to a 3% Synergel/Agarose gel

a. Subtract 0.7% from the 3% agarose; 3 - 0.7 = 2.3%
b. Divide the difference by 2; 2.3 ÷ 2 = 1.15%
c. 1.15% is the amount of Synergel added to 0.7% agarose to produce the functional equivalent of a 3% agarose gel.

Some Common Questions and Answers on Synergel

Q. What is the gelling temperature of a Synergel/Agarose gel?
A. The gelling temperature is determined by the agarose component of the binary gel and is essentially the same as that of the particular agarose selected for the gel.

Q. If the mixture is cooled to 65°C before pouring, is it still pourable?
A. If it is cooled before pouring there is no problem as long as the agarose being used gels at a lower temperature.

Q. Is a Synergel/Agarose gel strong enough for bands to be clipped and removed? Electroeluted?
A. Yes. Yes.

Q. Does Synergel still give improved results, such as DNA band separation, when used with low-melt agarose?
A. Yes.

Q. Can samples be extracted using a phenol-containing composition?
A. Yes, the same as they would for agarose.

Q. What percentage of Synergel gives the most significantly superior results when compared to agarose?
A. In the high sieving range of very small DNA fragments, 2-3% Synergel and 0.7-1.0% agarose is recommended. Separation of fragments 25bp and less have been reported.

Q. Does a combination of Synergel and low-melt agarose require a high temperature in order to melt? How high?
A. Synergel must be boiled to dissolve. However, the remelting and gelling temperature is determined by the agarose component.

Q. Would heating a DNA fragment in low-melt agarose plus Synergel cause a reaction between the polysaccharide and the DNA?
A. No, Synergel and agarose are comparably inert.

Q. Can Synergel be used in a system with borate buffers?
A. The borate-containing agarose-Synergel combination as a gel melt solution will be more viscous and should be poured slightly warmer. Aside from the viscosity, there would not be a problem. Use 1/2 strength TBE buffer described in the Maniatis protocol. Use 0.75% Synergel for excellent results in the 200bp range. (10 volts per cm). The increased viscosity is due to a pseudo-crosslinking phenomenon observed when relatively high concentrations of Synergel are used.

Q. How can I improve transfer results of genomic DNA?
A. Use electroblotting, it should give virtually total recovery.