SEQUAGEL™

Sequencing System

IN BLUE LAB

national diagnostics

- ***Sequencing Grade***
- ***Aldehyde Free & Biuret Negative***
- ***Stabilized for Long Shelf Life***
- ***Consistently Crystal Clear Gels***
- ***Ready-to-Use for Gel Casting Solutions (19:1 Acrylamide:Bisacrylamide) from 0 - 20%***

National Diagnostics' SequaGel™ Sequencing System is a ready-to-use system for the preparation of the identical nucleic acid sequencing gels that you are now accustomed to preparing. The new 3-part formulation is so flexible, any of the commonly used sequencing formulations can be prepared, quickly and easily, for any gel from 0-20% monomer.

FAST & EASY. With SequaGel™ there is no mess, no fuss, and no bother. The time-consuming process of weighing, mixing, and filtering different reagents to prepare stock solutions is eliminated. All you need do with SequaGel™ is pour and use. SequaGel™ allows you to follow the same procedures that you are presently using because it is identical to your own gel casting solutions. With SequaGel™, you can now prepare the same gels faster, easier, safer, and more reliably.

SAFE. SequaGel™ reduces exposure to neurotoxic acrylamide dust by eliminating the need for handling and weighing solid acrylamide and methylene bisacrylamide, which often leads to airborne particulate that can be readily inhaled. The vapor pressure of the SequaGel™ solution is so low that only water vapor escapes to the air.

RELIABLE. SequaGel™ is made stable by the incorporation of a gaseous inhibitor that prevents the initiation of the acrylamide and bisacrylamide. Only in this stable aqueous solution is the monomer prevented from the self-polymerization that solid acrylamide is susceptible to. Stabilization also prevents the accumulation of oxidation products such as acrylic acid. This gaseous inhibitor can then be completely removed during deaeration, prior to gel casting.

UNMATCHED PURITY. There is no comparable product available that is any purer. SequaGel™ is manufactured from the highest quality materials. All three solutions are formulated with distilled, deionized water, and then they are filtered to remove any additional impurities. The acrylamide and bisacrylamide have virtually zero acrylic acid contents. This eliminates fixed charges on cast gels, which can cause electroendosmosis and ion exchange effects that result in artifacts such as streaking. Thus band resolution is improved with SequaGel™, as confirmed by our test researchers across the country. In addition, oxidation products such as aldehydes are removed from solution by a selective adsorption process. Aldehydes can attack proteins or nucleic acid fragments, altering the structure of the sample, and thus altering R_f values. With SequaGel™, you can trust that your results will be consistent from one electrophoretic run to another. National Diagnostics' urea is always Biuret negative, and because Biuret is an accurate indicator of ammonia contamination, it is always ammonia-free. This guarantees reproducible separations without increases in ionic strength and conductivity. National Diagnostics' tris buffer is also free of ammonia and amine impurities, providing a stable pH.

REPRODUCIBLE RESULTS. SequaGel™ consistently produces crystal clear gels of the same molecular composition. Identical gel pore sizes guarantee consistent, reliable electrophoretic runs and R_f values time after time.

ECONOMICAL. Although National Diagnostics' SequaGel™ provides higher purity, reliability, and convenience, the cost of using SequaGel™ to prepare sequencing gels is no more expensive than making the solutions yourself. You can have all the advantages that SequaGel™ offers for the same price you are paying now, without having to waste valuable research time to make your own solutions.

Acrylamide and methylene bisacrylamide have been found to be neurotoxins. Protective gloves should be worn while handling these products as well as solutions of these products. In case of accidental ingestion, contact a physician immediately.

Storage: After opening, SequaGel™ Sequencing System is stable for 12 months when stored, tightly capped, in a dark area at room temperature (20° C).

SequaGel™ Sequencing System is supplied as a 3-part system. SequaGel Concentrate is supplied in 500ml and 1 liter bottles, containing the equivalent of 237.5 grams of acrylamide, 12.5 grams of methylene bisacrylamide, and 500 grams of urea (8.3M) per liter of solution. SequaGel Diluent is supplied in 500ml and 1 liter bottles, containing 500 grams of urea (8.3M) per liter of solution. SequaGel Buffer is supplied in 125ml and 250ml bottles containing 50% urea (8.3M) in 1.0M Tris-Borate-20mM EDTA Buffer pH 8.3 (10X TBE).

Order No. **EC-833** SequaGel Sequencing System Contains 1 bottle each of SequaGel Concentrate, Diluent, and Buffer.	500ml kit 1-3 1 liter kits 4-7 1 liter kits	$42/kit $76/kit $71/kit
Order No. **EC-830** SequaGel Concentrate	500ml 1-3 liters 4-7 liters	$27/btl. $50/l. $47/l.
Order No. **EC-840** SequaGel Diluent	500ml 1-3 liters 4-7 liters	$10/btl. $18/l. $16/l.
Order No. **EC-835** SequaGel Buffer	125ml 1-3 250ml 4-7 250ml	$ 6/btl. $10/btl. $ 8/btl.

Bottle usage
Conc Diluent Buffer
1 ℓ : 2 ℓ : 330 ml

The monomer percentage to be used, to prepare sequencing gels, depends upon the size of the nucleic acid fragments to be sequenced. The greater the number of nucleotides to be separated, the lower the acrylamide percentage that is needed. The following are typical formulations (100ml of casting solution) for polyacrylamide nucleic acid sequencing gels depending upon the DNA fragment size. The following gels will all contain a final concentration of 8.3M urea and 1X TBE.

DNA Fragment Size (in nucleotides)*	% Monomer	Volume of SequaGel to use
100-200	4	16ml SequaGel Concentrate 74ml SequaGel Diluent 10ml SequaGel Buffer
80-200	5	20ml SequaGel Concentrate 70ml SequaGel Diluent 10ml SequaGel Buffer
60-150	6	24ml SequaGel Concentrate 66ml SequaGel Diluent 10ml SequaGel Buffer
40-100	8	32ml SequaGel Concentrate 58ml SequaGel Diluent 10ml SequaGel Buffer
10-50	12	48ml SequaGel Concentrate 42ml SequaGel Diluent 10ml SequaGel Buffer
1-20	20	80ml SequaGel Concentrate 10ml SequaGel Diluent 10ml SequaGel Buffer

*Fron, Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY

The following formulas can be used to calculate the volume of SequaGel™ needed for your exact specifications.

$$V_c = \frac{(V_t)\ (X)}{25} \qquad V_b = 0.1\ (V_t)$$

$$V_d = V_t - (V_c + V_b)$$

V_c = Volume of SequaGel Concentrate to be used (ml),

V_b = Volume of SequaGel Buffer to be used (ml),

V_d = Volume of SequaGel Diluent to be used (ml),

V_t = Total volume of gel casting solution desired (ml), and

X = % gel desired.

EXAMPLE: To make 100ml of an 8% sequencing gel, calculate the SequaGel volumes to be added as follows:

$$V_c = \frac{(100)\ (8)}{25} = 32\text{ml SequaGel Concentrate}$$

$$V_b = 0.1\ (100) = 10\text{ml SequaGel Buffer}$$

$$V_d = 100 - (32+10) = 58\text{ml SequaGel Diluent}$$

METHOD OF USE

1. Calculate how much SequaGel Concentrate, Diluent and Buffer you need to make your gels by using the above chart or formulas. Pour the solution into an Erlenmeyer flask with a side-arm. (If the urea has precipitated out of solution, warm the solution in a 30° C water bath, and swirl until the urea redissolves.)
2. In most cases SequaGel will gel without degassing. However, if degassing is desired, use the following procedure:
   Add a stirring bar to the solution and stopper the flask. De-gas the solution under vacuum for 5 minutes while stirring on a magnetic stirrer. After deaeration, warm in the 30°C bath if necessary to redissolve the urea.
3. Add 0.8ml of 10% (w/v) ammonium persulfate for every 100ml of gel casting solution. Swirl gently to mix.
4. Add 40µl of TEMED for every 100ml of gel casting solution. Swirl gently to mix.
5. Pour the solution into the gel casting cassette. The gel should set in 6-10 minutes.

Make >125 cc for wedge spacers.

For Additional Information And Order Placement:

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