# TreviGel™ 500 Product Review And Applications Guide

TreviGel 500 is a unique resolving matrix for the separation of low molecular weight DNA. The ability to resolve small (< 50 to 1300) and very small (<20 bp) DNA molecules makes TreviGel 500 ideal for applications involving the Polymerase Chain Reaction (PCR). Combining the resolving power of polyacrylamide with the ease of use of agarose makes TreviGel 500 ideal for most molecular biology applications.


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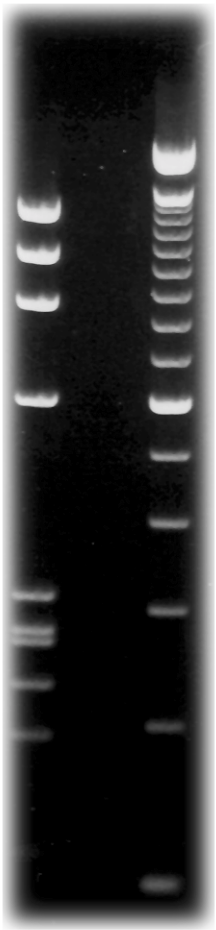


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## I. Preparation of TreviGel™ 500 Gels

TreviGel 500 is provided in powder form. The powder can be used to prepare horizontal gels for standard gel electrophoresis. Both TAE and TBE buffers may be used in casting the matrix and the choice of buffer depends upon the size of DNA to be resolved. TBE should be used when resolving very small DNA fragments (<50bp), however preparation of TBE gels is considerably more difficult. For TBE gels, refer to the "Advanced Protocols" section at the end of this document. Optimal performance is obtained by following a few simple steps when preparing the TreviGel. The following method for TAE gel casting gives consistent, high quality results.

TAE Gels:

The following TreviGel 500 concentrations are used based upon the size of DNA being resolved:

| Gel Concentration (%) | Recommended Size of DNA Separation (bp) |
|---|---|
| 0.5 | 300-1500 |
| 1.0 | 200-1000 |
| 1.5 | 150-800 |
| 2.0 | 50-600 |

**Electrophoresis Buffer**

**50X TAE**
2.0 M Tris pH 7.8
1.0 M Sodium Acetate
50 mM EDTA

1. Weigh out sufficient TreviGel 500 for the volume of gel required. For example, 100 ml of a 1 % gel is made by weighing out 1 gram of TreviGel 500 powder.

2. Prepare 1X TAE buffer for casting and running the gel. Add the 1X TAE buffer to a clean, dry Erlenmeyer flask (100 mls in this example), add the Trevigel powder to the buffer, and swirl until all the TreviGel is in suspension. Place the flask on a top loading balance, and tare or record the weight.

3. Microwave on medium to high setting until no particulates are visible in the molten gel. The time will vary, since microwave units vary in power. In general, TreviGel 500 requires microwaving for about a minute longer than agarose. Gently swirl flask to mix. HANDLE WITH CARE, the molten TreviGel 500 is very hot. Replace water that evaporated during microwaving by returning to top loading balance and adding distilled water until the tared weight (or recorded weight) is reached. Gently swirl to mix.

4. Once bubbles have stopped forming, wait an additional minute, and pour the gel into a casting tray. Ethidium bromide may be added just prior to pouring (0.25 µg/ml final concentration), however, this may affect relative DNA mobility. Allow the gel to cool for 30 minutes.

5. For optimal results, place gel at 4˚C for 30 minutes.

**Preparation Hints**

- TreviGel 500 can be prepared in 0.5 to 3.0% gels in either 0.5X or 1X TAE buffers (50X TAE = 2.0 M Tris pH 7.8, 1.0 M Sodium Acetate, 50 mM EDTA).
- For optimal performance, enough TAE buffer should be prepared for casting the gel and for use during electrophoresis. This makes the TAE concentration uniform between the gel and running buffer.
- Dirty glassware causes small bubbles to form within the molten TreviGel 500 which may be difficult to remove after casting the gel. Carefully clean the flask used to prepare the TreviGel 500.
- Add the TreviGel 500 to the buffer rather than adding the buffer to the TreviGel.

This will assure that all the TreviGel is in solution prior to microwaving. If small clumps of TreviGel are visible before microwaving, vigorously swirl the flask to disrupt them.

- To facilitate cleaning the gel material from the flask, immediately after pouring the gel, fill the flask with COLD water and swirl. The gel should solidify and form a single mass which is easily removed from the flask.

## II. TreviGel™ 500 Characteristics

### Physical Characteristics

- RNase/DNase Activity: No contaminating nuclease activity
- Electroendoosmosis: $\leq 4 \times 10^{-7}$ $cm^2/V \cdot S$
- Melting Temperature: Greater than 80˚C
- Remelting Temperature: 80˚ - 90˚ ± 2˚C
- Gelling Temperature: 37˚ ± 2˚C
- Staining Compatibility: Ethidium Bromide, SYBR® Green, SYBR® Gold

Typical separation of DNA fragments using TreviGel 500 in TAE buffer is shown in Figure 1. TreviGel showed improved resolution, clarity, and low background staining compared to a variety of competitive electrophoresis products.

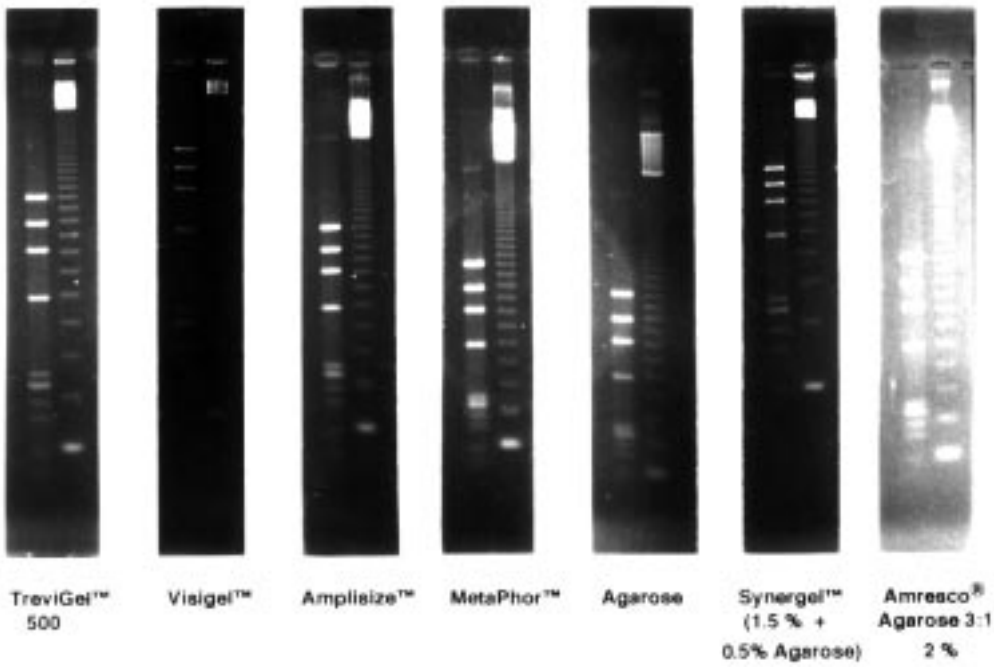



Figure 1. Typical separation of DNA fragments using TreviGel 500 and competitor products. To compare the DNA separation quality achieved using TreviGel 500 to competitive products, 1.5 % gels (except where noted in figure), were prepared in 1X TAE buffer according to the manufacturers instructions. The separation properties of TreviGel 500 meet or exceed those of the competitor products, giving a wider range of separation on one gel. For interpretation of molecular weight standard DNA fragment sizes used throughout this document, refer to the appendix.

### Clarity of TreviGel 500

The TreviGel 500 matrix forms a very clear gel with very low autofluorescence, allowing smaller amounts of DNA to be visualized and photographed. TreviGel 500 is about twice as clear as competitive gels.

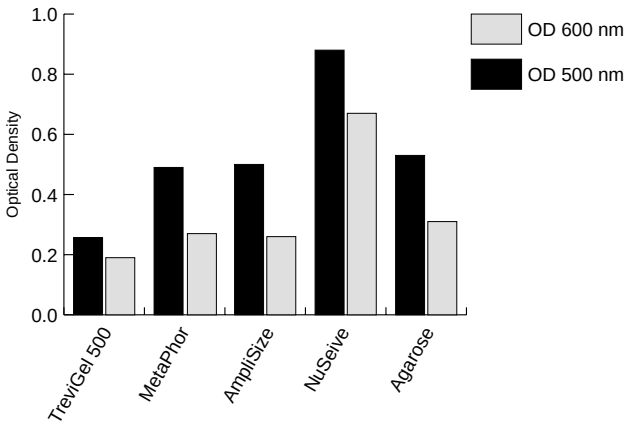



Figure 2: Clarity of the TreviGel™500 matrix, compared to competitor products. All gels are 2%, prepared in 1 X TAE buffer. Optical Densities were obtained by filling a 2 ml polycarbonate cuvette with 1.0 ml of TreviGel™500 or competitive product. Optical densities were obtained at either 500 or 600 nm using a Beckman DU-65 spectrophotometer. The graph above summarizes the data obtained from 3 independent readings.

## III. Electrophoretic Properties of TreviGel™ 500

TreviGel 500 is a clear gel matrix which enhances DNA visualization. Using optimal ethidium bromide concentrations (see section 1 below) it is possible to produce high quality photographic images with low background fluorescence. Also, the gels high melting temperature makes it possible to separate DNA fragments quickly by electrophoresis at very high voltage. Together, these features improve the quality of critical data presentation, without having to wait for the results.

### 1. Optimal Ethidium Bromide Staining Conditions Following Electrophoresis.

TreviGel 500 gels (2%) were loaded with 1 µg *Hae*III cut ΦX174 DNA. The DNA fragments were separated by electrophoresis at 75 volts for 1 hour in 1X TAE buffer. Following electrophoresis, gels were stained in 1 liter of either 0.1, 0.25 or 0.5 µg/ml ethidium bromide solution (ethidium bromide in distilled water) for 10, 20, or 30 minutes, respectively. Gels were photographed using Polaroid Type-57 film, and were evaluated for clarity and low background. Optimal staining was achieved using 0.25 µg/ml ethidium bromide for 15-20 minutes. Gels were not destained prior to photography. Shaking the gel in the ethidium bromide solution decreases the staining time by about 5 minutes. For non-critical electrophoresis and DNA quantitation, ethidium bromide may be added at a concentration of 0.25 µg/ml to the molten gel prior to casting. Ethidium bromide must also be used in the running buffer at the same concentration.

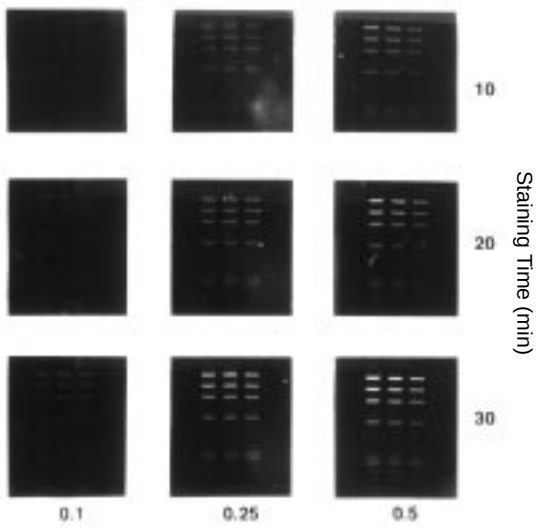
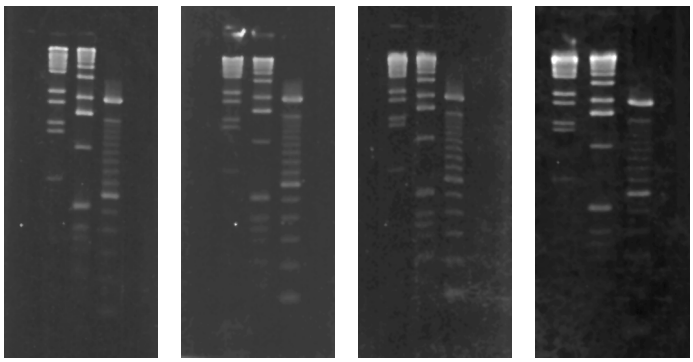



Figure 3. Optimal staining conditions of TreviGel 500. One microgram *Hae*III cut ΦX174 DNA was loaded onto 2% TreviGel™500 and separated by electrophoresis. Gels were stained in either 0.1, 0.25, or 0.5 µg/ml ethidium bromide, respectively. At 10, 20, and 30 minute time points (staining time), gels were removed from the stain and photographed.

### 2. Optimal Electrophoretic Conditions.

The high re-melting temperature (90˚C) of TreviGel 500 makes the matrix an effective rapid separation matrix because it can withstand the high temperatures generated by high voltage electrophoresis. Use of 0.5X TAE allows higher voltage electrophoresis since at the same voltage, the current is lower than with 1X TAE. Figure 4 shows the results of high voltage electrophoresis with 2% TreviGel 500, 0.5X TAE gels. The DNA was separated by electrophoresis at 100, 200, 250, or 400 volts. Buffer temperatures were monitored during the run, and final temperatures were recorded. DNA resolution began to suffer only at the highest voltages (400V), with buffer temperatures reaching 59˚C in 5 minutes at which point the run was completed. This experiment shows the utility of TreviGel 500 for rapid separation of small DNA fragments. A 12 minute run at 250 volts fully separated all DNA markers with little loss in resolution. It should be noted that optimal separation results will be obtained at lower rates of electrophoresis, or by running at lower temperatures, such as at 4˚C.



100 volts 200 volts 300 volts 400 volts

Figure 4. Separation of DNA fragments on TreviGel 500 at various voltages. To determine the effect of high voltage electrophoresis on resolution, λ-DNA cut with *BstE*II, 1 kb ladder, and 100 bp ladder were loaded onto 2 % TreviGel 500, 0.5X TAE gels. DNA was electrophoresed at 100, 200, 300, or 400 volts, respectively.

## IV. TreviGel™ 500 Applications

### 1. Southern Transfer of DNA from TreviGel™ 500.

TreviGel 500's gel strength and resolution makes it particularly useful for the purpose of DNA transfer, because it allows for high resolution probing of DNA sequences. The strength of the gel allows for easy handling of the gel during post electrophoretic manipulations. The high resolution allows the separation of small DNA fragments, not possible with standard agarose.

Three transfer methods were evaluated for their applicability to the TreviGel matrix. Gels were denatured (15 minutes in 1.5 M NaCl, 0.5 M NaOH) and neutralized (45 minutes in 1.5 M NaCl, 0.5 M Tris pH 7.5), prior to transfer. Capillary transfer was performed using either (i) 6 X SSC for 16 hours, or (ii) 0.1 M sodium phosphate buffer (pH 7.0) for 16 hours. Electrophoretic transfer was performed by (iii) semi-dry electroblotting using 0.3X TAE buffer for 40 minutes. Zetaprobe (BioRad) membrane was used for each procedure. Filters were baked for 30 minutes at 80˚C *in vacuo* as recommended by the manufacturer. Prehybridization (30 minutes) and hybridization (14 hours) were performed at 65˚C using a phosphate based hybridization solution (Church and Gilbert, 1984). The filters were washed at 65˚C.

Figure 5 shows that TreviGel 500 allows successful transfer of DNA to immobilizing membranes by a variety of methods, with improved resolution compared to agarose. Further, the gel matrix does not interfere with subsequent steps in the hybridization process. The Southern hybridization protocol is enhanced by the improved resolution of DNA fragments in TreviGel 500.

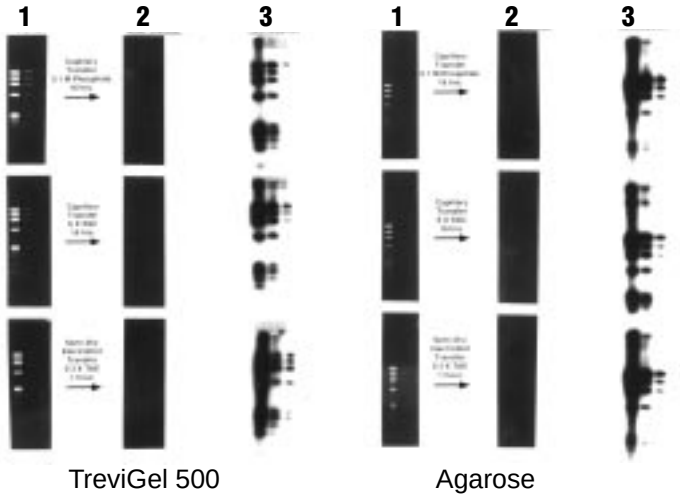



Figure 5. Southern Transfer of *Hae*III cut ΦX174 by capillary transfer (using either 6 X SSC or 0.1 M phosphate buffer pH 7.0), or by semi-dry electroblotting in 0.3 X TAE buffer. Results indicate a similar efficiency of transfer from each matrix (TreviGel 500, or standard Agarose). *Hae*III cut ΦX174 that was random prime radiolabeled with $^{32}P$ was used as the probe. Lanes 1 and 2 show ethidium bromide staining of gels before and after staining, respectively. Lane 3 shows autoradiograph of hybridized filters.

## 2. Preparation of DNA fragments from TreviGel™ 500.

The ability of TreviGel 500 to resolve small DNA fragments makes the matrix particularly attractive for the purpose of purifying small DNA fragments, or DNA of similar electrophoretic mobility. A number of methods have been developed to isolate DNA from agarose gels. We investigated the applicability of several of these methods to TreviGel 500. Figure 6 summarizes the relative efficiency by which DNA can be extracted from several matrices. TreviGel 500 allowed for almost identical recovery rates compared to other matrices under a variety of extraction conditions.

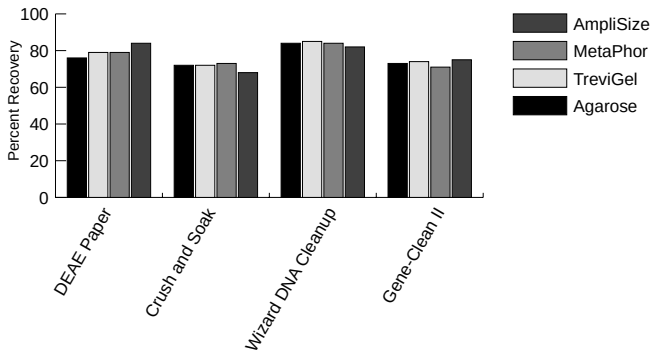



Figure 6. Percent recovery of DNA from several matrices. *Hae*III digest of ΦX174 was labeled using T4 polynucleotide kinase and γ-$^{32}P$-ATP. Labeled DNA (500,000 cpm) was loaded into each of 5 lanes of either a TreviGel 500, Metaphor™, Amplisize™, or Agarose gel (2% each in 1 X TAE buffer). The DNA was separated by electrophoresis, and the gels were exposed to autoradiographic film for 30 minutes to 1 hour. The 603 bp fragments were identified from the autoradiogram and excised from the gel with a clean razor blade. DNA was isolated from the gel by a variety of methods as per the manufacturers instructions. DNA recovery was quantitated using a Beckman LS6800 scintillation counter. Counts were compared to a standard unextracted fragment prepared by microwaving an excised agarose plug and placing it in scintillation fluid.

### a. Extraction Methods:

i) Transfer to DEAE paper. The fragment of DNA to be purified is excised from the TreviGel 500 matrix with a clean razor blade. The gel slice is embedded into 0.5% TreviGel 500 by casting a new gel around the excised fragment. After the cast gel has completely solidified, a razor blade incision is made approximately 0.5 to 1 cm from the gel slice in the direction of electrophoretic mobility. DEAE paper is cut to be slightly larger than the gel slice, wetted in electrophoresis buffer (either TAE or TBE) and placed in the razor incision, such that the path of electrophoresis will bring the DNA in contact with the paper. Electrophoresis is performed at 60 volts for 30 minutes. DNA can be removed from the paper by heating to 65°C in extraction buffer containing 1 M NaCl, 1 mM EDTA, 10 mM Tris pH 7.5 for 30 minutes, followed by precipitation with two volumes of ethanol. DNA is collected by centrifugation at 12,000 x g for 15 minutes.

Precipitation of DNA can be aided by the addition of 1 μg yeast tRNA to the sample prior to the addition of ethanol, as long as the tRNA will not hamper further processing of the DNA.

ii) Crush and Soak: The gel slice containing the DNA to be extracted is placed into a 1.5 ml microcentrifuge tube and crushed using a pipet tip, glass rod, or plastic pestle. Two gel volumes of TE buffer (10 mM Tris pH 7.5, 1 mM EDTA) is added to the tube. After several hours (4-16 hrs) the tube is spun, and the supernatant containing the DNA is collected. The DNA can be precipitated at this point by addition of 1/10 volume of 3 M Sodium Acetate pH 7.0, and two additional volumes of cold ethanol. The DNA is collected by centrifugation at 12,000 x g.

iii) Wizard™ DNA Clean-Up. The Wizard™ DNA clean up system (Promega, Madison WI) is one of several column based products that may be used for this purpose. Manufacturer's protocol was followed.

iv) Gene Clean® II. Gene Clean® II (Bio-101, Vista CA) is a silica based purification system in which the gel fragment is dissolved in NaI solution and adsorbed onto a silica matrix called glass milk. Following washing of the glass, the DNA is eluted into a small volume of either water or TE buffer, as per manufacturers instructions.

**b. Biological Evaluation of DNA extracted from TreviGel™ 500:**

Biological activity of purified fragments was determined by a variety of methods. Purified DNA was tested for the ability to be labeled using a random priming protocol, ligated using T4 DNA ligase, subcloned and transformed, and digested in a standard restriction enzyme reaction.

i) Random priming: The 1363 bp HaeIII fragment of ΦX174 DNA (purified from TreviGel 500, and competitor products) was quantitated by comparing ethidium bromide fluorescence intensity to a known quantity of DNA standards, following electrophoresis on a 1 % TreviGel 500 gel. An equivalent of 25 ng DNA was labeled using the random primer method. (Feinberg, A, and Vogelstein, B., 1983) Label incorporation was determined by counting 1/100th of the sample in a Beckman LS6800 scintillation counter. DNA was labeled to greater than 1 x $10^9$ cpm/μg DNA in each case.

ii) Subcloning: *Hind*III cut λ-DNA was separated on a 0.7 % TreviGel 500 gel. The 2000 base pair fragment was isolated and purified by the Wizard™ DNA clean-up method described above. The fragment was subcloned into phosphatase treated *Hind*III cut Bluescript®-KSII plasmid by standard methods, and introduced into *E. coli* JM109 cells. The 2000 bp fragment was purified from agarose and MetaPhor™, and subcloned for comparison. Ligation and transformation were not inhibited by any of the matrices tested. Plating 1/10th (100 μl) transformation reactions, produced approximately 5,000 colonies per plate for all ligation reactions. Using blue-white selection, over 95% of the subclones contained the 2000 bp insert. White colonies from each ligation reaction were picked and mini-prepped for analysis. In each case, digestion with *Hind*III (figure 7) released at least one copy of the 2000 bp fragment.

iii.) Restriction analysis of DNA fragments prepared from TreviGel 500 (figure 7). The 2000 bp λ-*Hind*III (0.5 μg) prepared from TreviGel 500, agarose, and MetaPhor™ were separately digested with 1 Unit of restriction enzyme *Afl*III for 1 hour at 37°C in a reaction volume of 25 μl. Following incubation, Orange-G (Cat. # 9850-250) was added, and the fragments were separated by electrophoresis on 1% TreviGel 5000 (Cat. # 9806-50-P).

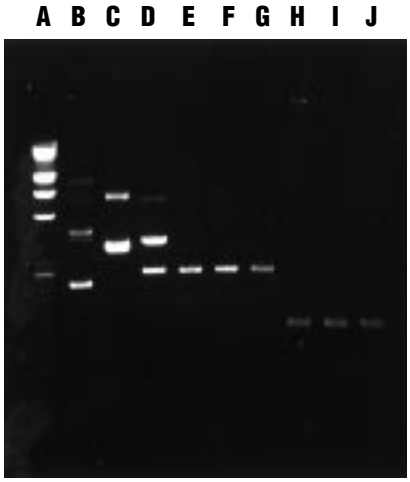



Figure 7. Biological analysis of DNA fragments isolated from TreviGel 500. DNA fragments were analyzed on a 1% TreviGel 5000 gel. A) λ-DNA *Hind*III fragments; B) Bluescript®II-KS vector-no insert; C) Mini-prep DNA from a TreviGel™500 isolated 2000 bp ligation/transformation; D) Mini-prep DNA cut with HindIII releasing the 2000 bp fragment; E) 2000 bp λ-DNA *Hind*III fragment purified from agarose; F) TreviGel™500; G) or MetaPhor™ respectively; H) 2000 bp λ-DNA *Hind*III fragment purified from agarose; I) TreviGel™500; J) or MetaPhor™, respectively, after digestion with restriction enzyme *Afl*III.

## V. Advanced TreviGel™ 500 Protocols

The TreviGel 500 matrix is very versatile. Described below are a number of specialized techniques that allow the researcher to fully exploit TreviGel 500's unique properties.

### 1. TBE Gels

**Electrophoresis Buffer**

**10X TBE**
1 M Tris pH 8.3
0.83 M Boric Acid
10 mM EDTA

The TreviGel 500 matrix is more viscous in TBE than in TAE buffer, making casting somewhat more difficult. However, resolution of very small DNA fragments is dramatically enhanced. Preparation of TreviGel 500 in 1X TBE (10X TBE = 1M Tris pH 8.3, 0.83 M Boric Acid, 10 mM EDTA) can be easily accomplished if care is taken. The strength and other properties of the gel are not altered when TBE is used. Choice of the lowest optimal concentration is critical, because the gel becomes very viscous during preparation. The appropriate TreviGel 500 concentration range for TBE gels is between 0.5 and 2%. The TreviGel should be prepared by microwaving in water then adding 10X TBE to the gel to a final concentration of 1X after microwaving. It is important to add the water to the flask BEFORE adding the TreviGel. This will prevent clumping of the gel. Also, water must be added back to the flask after microwaving to compensate for water lost during the heating process. Taring a balance with the flask, water and TreviGel 500 before heating, and adding back the appropriate mass of water after heating, is a quick way to accomplish this. Adding back water requires swirling the flask fairly vigorously, which may form small bubbles. After adding the TBE buffer, gently swirl to avoid adding bubbles. Unlike TreviGel 500 made in TAE, the gel made in TBE should be poured immediately after heating. Ethidium Bromide may be added to the molten gel (a concentration of 0.25 μg/ml is optimal), however, one must be aware that ethidium bromide may alter the relative electrophoretic mobility of DNA.

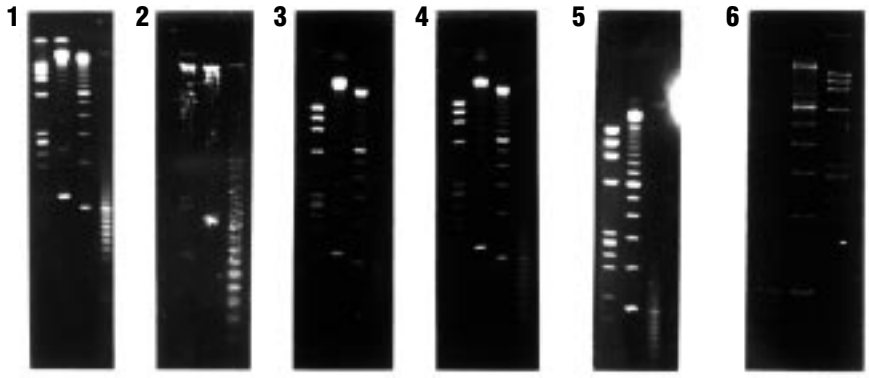



Figure 8. DNA separation by TreviGel 500 and competitors prepared using 1 X TBE buffer. One µg each ΦX-174 DNA cut with HaeIII, 123 bp ladder, 100 bp ladder, and 10 bp ladder (except where noted) were loaded onto 1) 2% TreviGel™500, 2) VisiGel™, 3) Amplisize™, 4) MetaPhor™, 5) agarose, and 6) 5% polyacrylamide. DNA's were separated by electrophoresis, stained for 20 minutes in 0.25 µg/ml ethidium bromide, and photographed. VisiGel™ is incompatible with TBE buffer, as evidenced by the smearing of DNA bands.

## 2. Electrophoretic Mobility Shift Assays

The electrophoretic mobility shift assay (EMSA) provides a rapid method for investigating the nature of protein:DNA interactions (Fried and Crothers, 1981). Current methods involve the use of polyacrylamide gels to separate bound from unbound forms of a protein:DNA binding complex. TreviGel™500 replaces polyacrylamide gels in this assay, giving identical results in a simplified and improved protocol.

Materials and Methods: HeLa nuclear extract and AP-1 consensus sequence oligonucleotide were used to investigate the applicability of TreviGel 500 to the EMSA. Double stranded AP-1 consensus sequence (shown below), was 5' end-labeled using T4 polynucleotide kinase to a specific activity of 2.8 X $10^9$ cpm/µg DNA (3.6 X $10^7$ cpm/pmol).

| | |
|---|---|
| AP-1 (c-jun) binding consensus sequence (AP-1 oligonucleotide) | 5'-CGCTTGATGAGTCAGCCGGAA-3'<br>3'-GCGAACTACTCAGTCGGCCTT -5' |

Labeled oligonucleotide (100,000 cpm) was incubated in 0.5 X Buffer D (1X Buffer D = 20 mM HEPES pH 7.9, 20 % glycerol, 100 mM KCl, 0.2 mM EDTA, 0.5 mM Dithiotreitol (DTT), and 0.5 mM Phenylmethylsulfonyl Fluoride (PMSF) (Dignam, *et al*., 1983)), in the presence of 1 µg poly(dI-dC):poly(dI-dC) and increasing amounts of HeLa nuclear extract, both in the presence and absence of 100 fold excess AP-1 double stranded oligonucleotide as specific competitor. The final reaction volume was 20 µl. Incubation was performed at room temperature for 30 minutes, after which time one half of the reaction (10 µl) was loaded directly onto each of a 2% TreviGel 500 horizontal gel or a 5% polyacrylamide vertical gel, each in 0.5 X TBE (refer to section describing casting TBE gels). The gel was cast in a 10 cm X 14 cm casting tray (total gel thickness was 3 mm). For comparison purposes, a 14 X 18 cm, 1.5 mm thick, vertical 5% polyacrylamide gel (20:1 acrylamide:bisacrylamide) was cast using 0.5X TBE.

Samples (10 µl) were loaded onto either the TreviGel 500, or polyacrylamide gels and separated by electrophoresis at 150 volts, until the unbound DNA was within one centimeter of the bottom of the gel, as determined by relative migration of Orange G tracking dye loaded into an empty well. Total electrophoresis time was about 1 hour for TreviGel 500, and 1.5 hours for the polyacrylamide gel. Following electrophoresis, the gels were dried with a vacuum gel dryer for 1 hour at 70°C and exposed to film for 4 hours. See Figure 9.

EMSA is often used as a first approximation in defining the components of a protein:DNA complex. The protocol outlined above enhances the assay by providing a simpler and safer method for the separation of bound and unbound forms of the complex. The method is fast, uses standard laboratory equipment, and offers the following improvements as compared to current protocols: First, the gel matrix is non-toxic, inexpensive, and can be easily cast in a horizontal format. Second, resolving properties of TreviGel 500 compare to those of polyacrylamide at the concentrations used for gel shift assays. Third, the strength of the gel provides the ability to cast very thin (< 4 mm) gels which can be further manipulated, and quickly dried at high (up to 80˚C) temperatures. Fourth, the horizontal format allows one to cast a single gel with multiple combs for the simultaneous processing of a great number of samples. Finally, the matrix allows for capillary transfer of the shifted complex to immobilizing membranes such as nitrocellulose or nylon, allowing further analysis of the shifted complex (*eg.* Western blot, or N-terminal sequencing), without the need for electrotransfer methodologies as required by polyacrylamide.

The AP-1:DNA complex in both gel matrices formed a single major band. A minor nonspecific band was detected in the polyacrylamide gel, and was also seen in longer exposures of the TreviGel™500 gel. Increasing the concentration of non-specific competitor was able to completely eliminate this minor band.

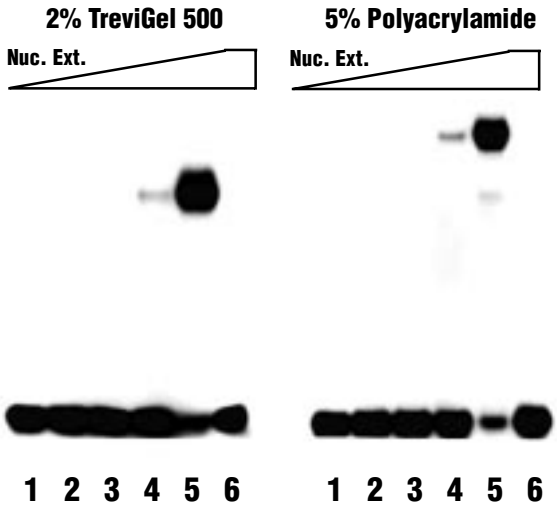
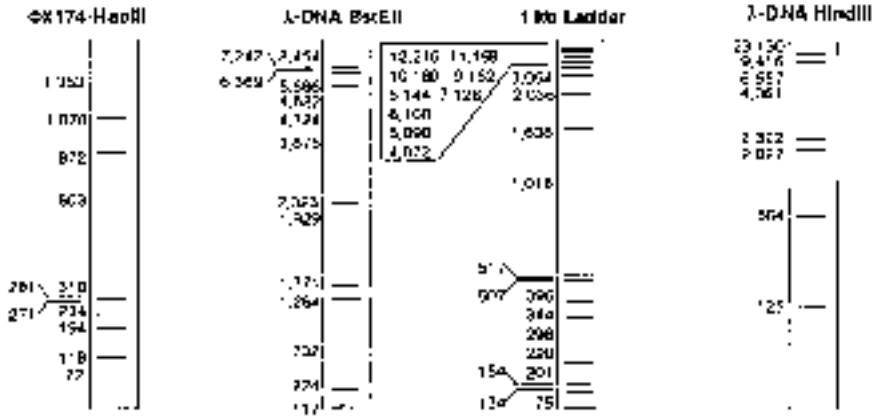



Figure 9. Side by side comparison of the electrophoretic mobility shift assay using either a 2% TreviGel 500, or 5% polyacrylamide gel. Lane 1, free AP-1 probe in the absence of nuclear extract. Lanes 2-5, increasing amounts of HeLa nuclear extract (0.2, 0.5, 1.0, and 5 µg, respectively) produce the single band specific to the AP-1:DNA complex (visible only in lanes 3-5). Lane 6 includes the addition of 100 fold excess unlabeled AP-1 oligonucleotide as a specific competitor.

The superior strength of the TreviGel 500 matrix allows for easy handling of very thin gels, making it a versatile, non-toxic alternative to polyacrylamide. The ability to cast and run the gel in a horizontal format expands and simplifies the EMSA protocol without sacrificing resolution of protein:DNA complexes separated by electrophoresis.

## VI. Appendix




Molecular Weight Standards Electrophoretic Migration. (Not Drawn to Scale)

Figure 10. Schematic representation of DNA molecular weight standards electrophoretic migration. Note: migrations are approximate and will depend upon the type and concentration of the gel. Higher molecular weight DNA bands (larger than 1500 bp) may not resolve on higher percentage gels.

| **Related Electrophoresis Products:** | **Catalog Number** |
|---|---|
| TreviGel 500, 50 g | 9804-50-P |
| TreviGel 500, 100 g | 9804-100-P |
| TreviGel 500, 250 g | 9804-250-P |
| TreviGel 5000, 50 g | 9806-50-P |
| TreviGel 5000, 100 g | 9806-100-P |
| TreviGel 5000, 250 g | 9806-250-P |
| Orange G Loading Buffer (5X) | 9850-250 |
| 50X TAE Buffer | 9860-500 |

## VII. References

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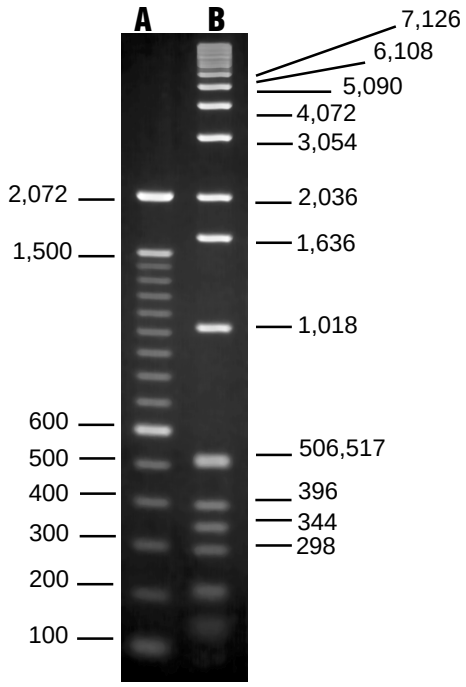
## TreviGel™ 5000

TreviGel 5000 is the perfect counterpart to TreviGel 500. This gel offers similar strength and clarity as TreviGel 500, yet resolves fragments in the 1000 to 25,000 bp range.




**1.2% TreviGel 5000**

A = 100 bp ladder
B = 1 kb ladder

2 µg DNA per lane

| Gel Concentration (%). | Recommended Range of DNA Separation (Kbp) |
|---|---|
| 0.5 | 2 - 25 |
| 0.6 | 1 - 20 |
| 0.7 | 0.8 - 10 |
| 0.9 | 0.5 - 7 |
| 1.2 | 0.4 - 6 |
| 1.5 | 0.2 - 3 |
| 2.0 | 0.1 - 2 |

TreviGel 5000 can be used for routine DNA gel electrophoresis. TreviGel 5000 powder is available in several sizes and includes easy-to-follow instructions for use. The powder is quickly and easily prepared by heating in TAE buffer. TBE buffer may also be used by heating the gel in water prior to adding the appropriate volume of 10X TBE buffer to make a final 1X concentration. TreviGel 5000 is clearer and stronger than regular agarose and provides sharper band resolution.





*The Polymerase Chain Reaction is protected by U.S. Patent Nos. 4,683,195 and 4,683,202 owned by Hoffman-LaRoche.