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ProductInformation

ACRYLAMIDE SOLUTION
for Mutation Detection

Product Number **A 5934**
Technical Bulletin No. MB-370

TECHNICAL BULLETIN

Product Description
Characterization of disease-causing mutations, identification of DNA polymorphisms, and detection of single-base substitutions requires sensitive screening methods. SSCP (single strand conformation polymorphisms) analysis[9,10] is effective in detecting genes with single base substitutions and polymorphisms.[6,10] The method involves amplification of a segment of genomic DNA by PCR* in the presence of radiolabeled nucleotides, melting and snap-cooling the strands of the PCR products, and analysis of the single strands in a non-denaturing polyacrylamide-based gel in TBE buffer.

The mobility of the single strands varies depending upon the amount of double stranded regions within the strand. A control lane with wild-type DNA should show two bands, each corresponding to one single DNA strand of the PCR product. Control mutant DNA should also show two bands, but each with shifted mobility compared to the wild-type control bands. A fainter band below these may be observed in each lane, corresponding to double-stranded, reannealed product. A non-denatured normal product control should be run on the gel to locate the nondenatured or renatured PCR product.

A heterozygous sample of DNA should demonstrate two bands identical to the mobility of the two strands in the normal sample and two bands identical to those in the mutant DNA. Additional bands may be observed as some strands may adopt more than one conformation. (The normal or wild-type sample should contain those bands corresponding to the different conformations.)

The sensitivity of SSCP for detecting mutations in conventional polyacrylamide gels is reported to range from 35% to almost 100%.[8,9,12] Maximal sensitivity is often accomplished by optimizing running conditions, such as adding glycerol to gels, reducing the run temperature, and increasing the length of the gels and the run time.

Sigma's Mutation Detection Acrylamide gels significantly improve resolution of DNA molecules differing in conformation compared to conventional polyacrylamide gels. Enhanced sensitivity is often achieved in the Mutation Detection Acrylamide gels without optimizing running conditions. Even so, the success of any SSCP experiment depends upon the DNA fragments being evaluated and optimization of experimental conditions to increase the differential migration between the DNA strands. The optimal sensitivity of SSCP is found with amplicons of 200 or fewer nucleotides in size.

Sigma's acrylamide-derived matrix for SSCP analysis requires a sequencing apparatus with 0.4 mm spacers and combs. The optimal electrophoretic conditions for a specific set of templates and primers is determined empirically. Under comparable buffer, temperature and electrophoretic conditions in SSCP experiments, DNA strands will run somewhat more slowly on Sigma's Mutation Detection gels than on standard 6% polyacrylamide gels.

Reagents

- 2 X Acrylamide Solution for 250 ml Mutation Detection, Product No. A 5934

Reagents Required But Not Provided
(Sigma Product Numbers have been given where appropriate.)

- Sequencing apparatus
- Power supply capable of 1200 V or more
- Heat/vacuum gel dryer
- SigmaCote®, Product No. SL-2 or similar anti-stick product
- Ammonium persulfate, Product No. A 9164
- TEMED, Product No. T 7024
- 10X TBE, Product No. T 4415
- Stop Solution:
  95% Formamide, Product No. F 9037
  10 mM NaOH
  0.25% Bromophenol Blue, Product No. B 5525
  0.25% Xylene Cyanol, Product No. X 4126

Precautions and Disclaimer
Sigma's Mutation Detection gel solution is for laboratory use only. Not for drug, household or other uses. Wear gloves and use all safety precautions routinely used when handling acrylamide solutions. When radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed.

Storage/Stability
Store at room temperature. Sigma's Acrylamide Solution for Mutation Detection may be used until recommended date on the label.

Procedure
The procedure described below is suitable for detecting SSCP. Typically, fragments of 200 nucleotides or less are used for optimal sensitivity. Optimal running conditions depend largely on factors such as the size of the PCR fragment, location of mutations in the fragment, and base sequence of the fragment. Therefore, conditions must be optimized for each sequence.

Preparation of PCR-SSCP Samples

Use PCR conditions which minimize unwanted side products, as these can result in artifact bands which interfere with the identification of SSCP bands. Following are several important factors to consider for the best SSCP results:

a. Use only highly purified, salt-free template DNA.
b. Use primers with no partial mismatches in the target sequence.
c. Optimize reagent and primer concentrations for each amplification reaction.
d. Determine thermal cycle settings which eliminate non-specific priming.
e. Use the minimum number of PCR cycles to obtain a sufficient quantity of DNA, usually 30 cycles (or fewer) for 100 ng of genomic DNA.

The products of amplification reactions are routinely evaluated for purity by electrophoresis in highly resolving agarose gels. PCR artifact products which differ by 10-20 base pairs from the expected product (size range 100-400 bp) can be resolved on wide range/standard 3:1 agarose gel (4%) (Product No. A7431). PCR artifact products which differ by 3-10 bp from the expected product can be resolved on a high resolution agarose gel (4%) (Product No. A 4718). These artifact bands cannot be detected on standard agarose gels. The artifacts will be detected in Mutation Detection gels and may interfere with the identification of heteroduplex bands. Therefore, it is critical to use PCR conditions which minimize unwanted side products.

Gel Preparation and Pouring

1. Glass plates must be clean and free of dried gel and soap residues. To remove residues, apply ethanol to both plates and wipe dry.

2. Ensure that the gel will not stick to the glass plates by treating one plate with SigmaCote®, Product No. SL-2 or similar anti-stick product. If plates were previously silanized, the coating must be removed completely prior to retreatment.

3. Assemble glass plates according to the manufacturer's instructions. Use 0.4 mm spacers.

4. Acrylamide Solution for Mutation Detection is supplied as a 2X liquid concentrate. Table 1 lists the components required for 100 ml of 0.5X prepared gel solution. Adjust according to your plate volume.

Note: The selection of 0.5X concentration was the result of research performed on a specific set of mutations on the p53 tumor suppressor gene. The optimal concentration for each sample may vary. If you are not initially successful using 0.5X gel solution, you may wish to evaluate a concentration range from 0.4X to 1X.

Table 1
Preparation of 0.5X Mutation Detection Gel (100 ml volume)

2X Mutation Detection Gel Solution	25.0 ml
10X TBE	6.0 ml
Deionized water (for final volume of 100 ml)	68.6 ml
TEMED	40 ml
10% Ammonium persulfate (prepare fresh)	400 µl
Final volume	100 ml

5. Place the specified quantity of the first three components into a clean beaker and mix gently by swirling.
6. Add the specific amounts of TEMED and ammonium persulfate and mix.

7. Pour the gel solution into the plates using the standard procedure for acrylamide. If the gel contains a few bubbles, remove them with a piece of wire. Insert comb (in an inverted position if using a sharktooth comb) and allow the gel to polymerize for at least 60 minutes at room temperature.

8. Remove the comb, and rinse the wells or top surface of the gel well with 1X TBE buffer.

9. Mount the gel cassette onto the sequencing apparatus according to the manufacturer's instructions.

10. Prepare sufficient quantity of running buffer to fill both anode and cathode chambers by diluting 10X TBE stock to 0.6X.

Electrophoresis

1. After thermal cycling, add 1 µl of the radiolabeled PCR product to 10 µl of stop solution, heat to 94 °C for 2 minutes, and place the denatured DNA on ice.

2. Rinse the top of the gel thoroughly with running buffer. Reinsert sharktooth comb so it just touches the gel, and load 1-3 µl of sample.

3. Run the samples through the Mutation Detection gel at 20 v/cm for 14 hours at room temperature.

Autoradiography

1. When the run is complete, turn off the power supply, disconnect electrodes and remove the plates from the apparatus.

2. Allow the plates to cool briefly before separating. Transfer the gel onto medium thickness (3 mm) filter paper (Product No. P9039).

3. Place the paper on a flat surface with the gel side up and cover with plastic wrap.

4. Dry the gel and expose to X-ray film using standard techniques.

Results

The control lane with wide-type (normal sequence) molecules should show two bands each corresponding to one single DNA strand of the PCR product. A control mutant DNA will also demonstrate two bands, but with shifted mobilities compared to the normal bands. A fainter band below these may be observed, corresponding to double-stranded reannealed product. A non-denatured normal product should be run on the gel to identify this undenatured/renatured position.

A heterozygous mutation should be characterized by the presence of two bands identical to those in the normal sample and two bands characteristic of each strand of the mutant molecule. Additional bands may also be observed, because some strands can adopt more that one conformation. In this instance, the normal lane would then contain additional bands corresponding to the different conformations.

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