Peptide Mapping

Peptide mapping involves controlled cleavage of a pure protein with small amounts of a pure protease to generate peptides of characteristic, reproducible sizes. These peptides can be separated on PAGE to produce a "fingerprint" characteristic of the protein. Peptide mapping can map cleavage sites in an unknown protein, or it can identify an unknown protein based upon its fingerprint identity with a previously tested sample. The polyacrylamide gel used can be either denaturing or non-denaturing, but SDS PAGE is most often used because it gives molecular weight information about the peptides produced. Small amounts of protease are used, so that minor variations in time and temperature of incubations will not overly perturb the results. Proteins for peptide mapping can be taken from bands sliced out of electrophoresis gels, or purified by standard means. Protocols are provided for the mapping of a pure protein, a protein in an acrylamide gel, and a protein isolated from National Diagnostics' ProtoPrep II Matrix.

Peptide Mapping - Purified Protein

1. Dissolve protein to 0.5mg/ml in digestion buffer and heat to 100°C for 2 minutes.

   Digestion Buffer: 0.125M Tris HCl pH 6.8
   0.5% SDS
   10% glycerol
   0.0001% Bromophenol Blue

2. Cool to 37°C and digest with protease for 30 minutes (see table below for enzymes and amounts).

3. Stop digestion by adding SDS to 2% (1/10 vol of 20% SDS) and 2-Mercaptoethanol to 10% and heating to 100°C for 2 minutes.

4. Load 10 - 15 - 20 µl (5 -10 µg) on a 10 - 15% SDS PAGE gel for analysis.

<table>
<thead>
<tr>
<th>Proteases for Use in Peptide Mapping</th>
<th>Procease</th>
<th>Final Conc. (µg/ml)</th>
<th>pH optimum</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>5-20</td>
<td>7.9</td>
<td>Arg, Lys</td>
<td></td>
</tr>
<tr>
<td>Pepsin</td>
<td>10-20</td>
<td>6.7</td>
<td>Arg,Lys, Gln,His, Gly, Tyr</td>
<td></td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>50-100</td>
<td>7.8</td>
<td>Aromatic</td>
<td></td>
</tr>
<tr>
<td>Elastase</td>
<td>50-100</td>
<td>7.9</td>
<td>Uncharged Aliphatic</td>
<td></td>
</tr>
<tr>
<td>Staph. Aureus ProteaseV8</td>
<td>50-100</td>
<td>4.8</td>
<td>Asp, Glu</td>
<td></td>
</tr>
</tbody>
</table>

Peptide Mapping - Protein in a Gel Slice

1. Stain and destain as quickly as possible to avoid acid hydrolysis artifacts.
2. Cut out band (containing 1-10µg of protein) of interest and rinse slice in cold deionized water. Cut slice to the width of a well, as it will be loaded into the analytical gel.

3. Soak slice in 1X stacking gel buffer and 1 mM EDTA.

4. Place slice at the bottom of a well in the analytical gel (use a spatula or loading tip to place slice)

    **NB:** The analytical gel for this protocol must have a stacking gel of at least 3 cm, to allow a space for digestion to occur.

5. Overlay slice with stacking gel buffer + 20% glycerol.

6. Overlay this with 10 ml of stacking gel buffer + 10% glycerol + protease + 0.01% Bromphenol Blue

7. Run samples into stacking gel. When the tracking dye reaches the bottom of the stacking gel, turn off voltage for 30 minutes to allow digestion to occur. Then continue run as usual.

    *Note that smaller amounts of radiolabeled protein can be analyzed by this method.*

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**Peptide Mapping - Using ProtoPrep II**

*ProtoPrep II, National Diagnostics’ meltable acrylamide matrix, may be used to improve the convenience and reproducibility of peptide mapping. Proteins for mapping are first purified on a ProtoPrep II gel, and the bands cut out and melted. The protein is then digested in the melt, and loaded onto an analytical gel for mapping.*

1. **Prepare a ProtoPrep II gel.**

2. Load sufficient protein to have at least 1µg in the band of interest.

3. **Run the gel.**
4. **Stain gel** briefly with **Coomassie Blue R-250**.

   *NB: for optimal recovery of low levels of protein, run parallel lanes, stain one lane and use as a guide to excise protein from the unstained lane. The denaturing effect of protein stains may significantly reduce the recovery of some proteins.*

5. Cut out band of interest, trim band carefully to its minimum size.

6. Determine band weight by placing it in a pre-weighed microcentrifuge tube. (1mg=1µl)

7. To each slice add 2 - 3 volumes of ProtoPrep II Dissolution Reagent, and heat to 65°C until melted (1-2 hours).

8. Add 2 slice volumes of digestion buffer (see protocol above), containing desired protease. Incubate 30 minutes.

   *NB: The pH of this digestion is 0.5 units higher than that of the preceding protocols. This should not present a difficulty because most proteases will be more active at this pH.*

9. Add 0.1 vol **glycerol** and load on analytical gel.

Analytical gels for peptide mapping:
The choice of gel system for the analysis of peptide mapping is dictated by the anticipated results. If a wide range of peptide sizes is anticipated, a **gradient gel** may be required. For peptides over 7kd, standard **Tris Glycine SDS PAGE** gels will give superior results. Small peptides will require **strongly denaturing fixatives to avoid loss of signal** during staining. For extremely small peptides, analysis on **native PAGE gels** may be superior. In native protein PAGE, separation is based partly on charge to mass ratio. This can enable the resolution of peptides that would run too close to the SDS/dye front in SDS PAGE.

NEXT TOPIC: **Protein Purification using SDS-PAGE**

Products Related to this Discussion:

- **ProtoGel (30%)**
  30% concentrated solution of acrylamide and bis-acrylamide, 37.5 : 1 ratio. Filtered, deionized, and stabilized.

- **ProtoGel (40%)**
  Concentrated solution of acrylamide and bis-acrylamide, 37.5 : 1 ratio. Filtered, deionized, and stabilized.

- **ProtoGel Quick-Cast 12%**
  Ready-to-use gel solution for SDS-PAGE that combines the convenience of pre-cast gels with the economy
of casting your own.