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## Product Information

**ProteoPrep™ TRIBUTYLPHOSPHINE and  
ALKYLATING REAGENT, IODOACETAMIDE**  
Product Numbers **T 7567** and **A 3221**  
Store at 2-8 °C

### TECHNICAL BULLETIN

#### Product Description

Sigma's ProteoPrep Tributylphosphine and Alkylating Reagent are designed for the reduction and alkylation of disulfide bonds. Protein solutions can be treated with these reagents prior to gel electrophoresis so that artifactual disulfide bond formation does not occur during two dimensional gel electrophoresis. This results in less streaking and better resolution on two-dimensional electrophoresis gels.

Enough of each reagent is packaged to generate at least ten 2-ml samples. The reagents are individually packaged in ten vials each to ensure their freshness when opened. These two reagents are offered as separate product numbers so they can be purchased individually according to the user's particular application.

#### Components

Tributylphosphine Stock Solution, 10 x 0.5 ml flame sealed ampules, Product No. T 7567

Alkylating Reagent, Iodoacetamide, 10 x 56 mg in brown glass vials, Product No. A 3221

#### Reagents and Equipment Required But Not Provided

- micropipettors
- microcentrifuge tubes
- microcentrifuge
- High purity water (Product No. W 4502)

#### Precautions and Disclaimer

Not for *In vitro* diagnostic use.

#### Preparation Instructions

Tributylphosphine (TBP) Stock Solution. This reagent is a ready to use solution of TBP (200 mM) stored under argon in a flame sealed ampule. Once the ampule is opened the unused material can be stored up to 2 weeks if placed into an airtight glass vial, purged with nitrogen or argon and kept at -20 °C. This stock solution must be diluted 1:40 into the protein sample (25 µl of TBP stock solution into 1 ml of sample).

#### Alkylating Reagent, Iodoacetamide.

The Alkylating Reagent, Iodoacetamide solution should be made fresh just prior to use. Resuspend the contents of one vial of Product A 3221 with 0.6 ml of high purity water. Mix well until the entire solid has dissolved. This will make a 0.5 M stock solution. 30 µl of this stock solution should be added to every ml of sample. Discard any remaining material since it degrades quickly once it has been dissolved.

#### Storage/Stability

These reagents should remain stable for at least 6 months in their unopened container.

#### Procedure

1. Protein solutions are reduced by adding TBP to a final concentration of 5 mM (25 µl of TBP stock solution per ml of protein solution)
2. The sample should then be incubated at room temperature for 1 hour.
3. The protein solution is alkylated with 15 mM iodoacetamide (30 µl of Alkylating Reagent, Iodoacetamide per ml of protein solution)
4. Incubate the mixture for 1.5 hours at room temperature.
5. Centrifuge the final reduced and alkylated sample at 20,000 x *g* for five minutes at room temperature (microcentrifuge) to pellet any insoluble material.
6. This material is now ready for loading onto IEF gels. It is also suggested that the protein concentration be measured so that the amount of protein loaded onto the gels is known.

**References**

1. Molloy, M.P., et al., Electrophoresis, **19**, 837-844 (1998).
2. Herbert, B.R., Electrophoresis, **19**, 845-851 (1998).

Technology developed in partnership with Proteome Systems

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