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Product Information

Iodoacetamide and Tributylphosphine solution

Catalog Numbers **A3221 and T7567**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Sigma's Tributylphosphine solution and Iodoacetamide are designed for the reduction and alkylation of disulfide bonds, respectively. Protein solutions can be treated with these reagents prior to gel electrophoresis so 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 Solution (Catalog Number T7567)
10 × 0.5 ml flame sealed ampules

Iodoacetamide (Catalog Number A3221)
10 × 56 mg in brown glass vials

Reagents and Equipment Required But Not Provided

- micropipettors
- microcentrifuge tubes
- microcentrifuge
- Ultrapure water (Catalog Number W 4502)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, *In vitro* diagnostic, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Tributylphosphine (TBP) 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).

Iodoacetamide

An iodoacetamide solution should be made fresh just prior to use. Resuspend the contents of one vial of Catalog Number with 0.6 ml of ultrapure 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

Store the products at 2–8 °C.

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 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 × *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 also suggested 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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