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Product Information

ProteoPrep® Reduction and Alkylation Kit

Catalog Number **PROTRA**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The ProteoPrep® Reduction and Alkylation Kit contains tributylphosphine (TBP) and iodoacetamide (IAA), which are used for the reduction and alkylation, respectively, of protein disulfide bonds. The reduction and alkylation of proteins in solution may be performed on the sample prior to loading the immobilized pH gradient (IPG) strip, during equilibration of the focused IPG strip prior to SDS-PAGE electrophoresis, or in-gel prior to tryptic digestion. Treating the protein sample prior to loading the IPG strip is recommended;[1] this results in a 2D gel with less streaking, increased resolution, fewer artifacts, and better reproducibility. Although three procedures are supplied, protein samples need only be reduced and alkylated once.

Reagents

Tributylphosphine Stock Solution (Catalog Number T7567), 5 flame-sealed ampules each containing 0.5 ml of 200 mM TBP in 1-methyl-2-pyrrolidone packaged under argon. This solution is ready to use.

Alkylating Reagent, Iodoacetamide (Catalog Number A3221), 5 brown glass vials of 56 mg each.

Products Required But Not Provided

- ProteoGel™ IPG Equilibration Buffer (Catalog Number I7281)
- equilibration tray
- rocker (Catalog Number Z367745)
- Trypsin Profile IGD Kit (Catalog Number PP0100)
- Proteomics Grade Trypsin (Catalog Number T6567)
- ammonium bicarbonate (Catalog Number A6141)
- Biotech grade acetonitrile (Catalog Number 494445)
- trifluoroacetic acid (Catalog Number T6508)
- ultrapure water (18 MΩ·cm or equivalent)
- flat nosed tweezers
- siliconized microtubes (Catalog Number T4691 or equivalent)
- 37 °C heating block or heating bath
- scalpel (Catalog Numbers S2771 and S3021) or razor blade
- bench-top centrifuge (microcentrifuge)
- centrifugal concentrator (SpeedVac®)
- sonic bath
- ZipTip® pipette tips

Precautions and Disclaimer

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

Preparation Instructions

Tributylphosphine (TBP) Stock Solution - This reagent is a ready-to-use solution.

Alkylating Reagent (IAA) Stock Solution - The stock solution of the alkylating reagent, iodoacetamide, should be prepared just prior to use. Dissolve the contents of one vial in 0.6 ml of ultrapure water. Mix briefly until the solid is dissolved. The Alkylating Reagent Stock Solution is 0.5 M upon reconstitution.

Storage/Stability

Store the reagents as supplied at 2–8 °C. The unopened reagents should remain stable for at least one year.

Tributylphosphine (TBP) Stock Solution (Catalog Number T7567) - Once an ampule is opened, the unused material can be stored for up to 2 weeks when placed in an airtight glass vial purged with an inert gas and stored at –20 °C.

Alkylating Reagent (IAA) Stock Solution - Discard any remaining solution after use, since it degrades quickly once it is dissolved.

Procedures

Procedure for reduction and alkylation of the protein sample prior to loading the IPG strip

1. Prepare a sample with the proteins of interest using a solubilization reagent, such as Protein Extraction Reagent Type 4 (Catalog Number C0356), resulting in a solution with a pH >9.
   Note: Crude protein samples containing 5–10 mg of protein/ml work well with this solution procedure. Higher concentrations of protein may result in incomplete reduction or alkylation. The concentration of disulfides must be less than 2 mM. The pH of the solution being used in the reactions should be >7.5.
2. Reduce the protein sample by adding 25 µl of the TBP Stock Solution per 1 ml of protein solution. The final concentration of TBP is 5 mM. Incubate the sample at room temperature for 30 minutes. The protein solution will become hazy upon addition of the TBP Stock Solution. The solution will clear upon incubation.
3. The protein solution is then alkylated by adding 30 µl of the IAA Stock Solution per 1 ml of protein solution. The final concentration of iodoacetamide is 15 mM. Incubate the sample at room temperature for 1 hour.
4. Quench the excess iodoacetamide with additional TBP Stock Solution, since iodoacetamide may react with other amino acids during prolonged incubation or storage. Add 25 µl of the TBP stock solution per 1 ml of protein solution and incubate the sample at room temperature for 15 minutes.
5. Centrifuge the reduced and alkylated protein sample at 20,000 × *g* for five minutes at room temperature to pellet any insoluble material. The protein sample is now ready for loading onto an IPG strip.

Procedure for reduction and alkylation of proteins in solution during equilibration of a focused IPG strip prior to SDS-PAGE electrophoresis

1. Place the focused IPG strip in a plastic equilibration tray or plastic tube with the backing against the plastic surface. Equilibrate each strip separately.
2. Pipette the volume (see Table 1) of ProteoGel IPG Equilibration Buffer (Catalog Number I7281) or other suitable equilibration buffer over the strip. Ensure the volume is enough so the strip is completely covered.

Table 1.
Volume of Equilibration Buffer

Strip Length	Volume/Strip
7 cm	3 ml
11 cm	5 ml
18 cm	8 ml

3. Add TBP to the equilibration buffer to a final concentration of 5 mM (25 µl of TBP Stock Solution per 1 ml of equilibration buffer).
4. Place the tray or tube(s) on a rocker such that the strip is moving freely in solution and incubate the IPG strip at room temperature for 15 minutes.
5. Remove the equilibration buffer containing the reducing agent.
6. Pipette a fresh volume (see Table 1) of equilibration buffer over the IPG strip.
7. Add IAA to a final concentration of 15 mM (30 µl of the IAA Reagent Stock Solution per 1 ml of the equilibration buffer).
8. Place the tray or tube(s) on a rocker such that the strip is moving freely in solution and incubate the IPG strip at room temperature for 20 minutes.
9. Take the IPG strip out of the tray or tube and blot the backing of the IPG strip on a dry paper towel to drain the excess equilibration buffer. The IPG strip is ready for SDS-PAGE electrophoresis.

Procedure for reduction and alkylation of proteins during in-gel tryptic digestion[2]

The following procedure starts with a Coomassie® Brilliant Blue, SYPRO® Orange, or SYPRO Ruby stained 1D or 2D polyacrylamide gel. For silver stained gels, a gel destaining step different than that used for dye stained gels is required. The ProteoSilver™ Plus Silver Staining Kit (Catalog Number PROTSIL2) is recommended for silver staining prior to tryptic digestion and MS analysis. It contains destaining solutions for silver stained gels and a procedure for preparing gel slices for tryptic digestion.

1. Carefully cut the band of interest from a 1D gel or the protein spot from a 2D gel, using a scalpel or razor blade, taking care to include only stained gel. Lift out the gel piece using clean flat nosed tweezers.
2. Place the gel piece in a siliconized microtube. A siliconized tube reduces binding of the peptides to the tube surface. If unsure of chemicals leaching from the tube, which could interfere or suppress the MALDI-MS signal, prewash the tube with 100 µl of a 0.1% trifluoroacetic acid in 50% acetonitrile solution and then allow it to dry before use.
   Note: The gel piece may be cut into equal sections of 1–1.5 mm size and the sections may be used in place of the intact piece.
3. Cover the gel piece with 200 µl of 200 mM ammonium bicarbonate with 40% acetonitrile and incubate at 37 °C for 30 minutes. Remove and discard the solution from the tube.
4. Repeat Step 3 one more time.
5. Dry the gel piece in a Speed Vac for 15–30 minutes.
6. Prepare a fresh 20 mM TBP solution by diluting the TBP Stock Solution 10-fold with 25 mM ammonium bicarbonate. Add 100 µl of the 20 mM TBP solution to the sample tube and incubate the gel piece for 15 minutes at 37 °C with shaking.
7. Remove the supernatant. Prepare a 40 mM IAA solution by diluting the IAA Stock Solution 12.5-fold with 25 mM ammonium bicarbonate. Add 100 µl of the 40 mM IAA solution to the sample tube and incubate the gel piece for 30 minutes at 37 °C with shaking.
8. Remove the supernatant. Wash the gel piece with 200 µl of 25 mM ammonium bicarbonate for 15 minutes at 37 °C.
9. Remove the supernatant. Repeat the wash with 200 µl of 25 mM ammonium bicarbonate.
10. Wash the gel piece a third time with 200 µl of 25 mM ammonium bicarbonate in 50% acetonitrile for 15 minutes at 37 °C.
11. Dry the gel piece in a SpeedVac for 15–30 minutes.
12. The reduced and alkylated protein present in the gel piece can then be digested with trypsin (Catalog Numbers T6567 and PP0100, Proteomics Grade Trypsin and Trypsin Profile IGD Kit, respectively) and the peptide fragments eluted for subsequent MS analysis.

Related Products	Catalog Number
ProteoPrep Kits	
Total Extraction Sample Kit	PROTTOT
Membrane Extraction Kit	PROTMEM
Universal Extraction Kit	PROTTWO
Protein Extraction Reagent Type 4	C0356
Protein Extraction Reagent Type 1	C0481
Protein Extraction Reagent Type 2	C0606
Protein Extraction Reagent Type 3	C0731
EZBlue™ Gel Staining Reagent	G1041
ProteoSilver Plus Staining Kit	PROTSIL2
Bradford Reagent (recommended for 1–1,400 μg/ml protein)	B6916
BCA Kit for Protein Determination (recommended for 200–1,000 μg/ml protein)	BCA1
QuantiPro™ BCA Assay Kit (recommended for 0.5–30 μg/ml protein)	QPBCA
ProteoMass™ MALDI-MS Calibration Kits	
Protein and Peptide	MSCAL1
Peptide	MSCAL2
Protein	MSCAL3

References

1. Herbert, B.R., Electrophoresis, **19**, 845-851 (1998).
2. Speicher, K. et al., J. Biomolecular Techniques, **11**, 74-86 (2000).
3. Gorg, A., Two-Dimensional Electrophoresis of Proteins Using Immobilized pH Gradients. A Laboratory Manual. Technical University of Munich: 1998.
4. Molloy, M.P., et al., Electrophoresis, **19**, 837-844 (1998).
5. Galvani, M. et al., Electrophoresis, **22**, 2066-2074 (2001).

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