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Product information

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2D Sample Extraction Buffer

Catalog #: PL037 / PL038 / PL039 / PL040 / PL041 / PL042
Size: 50ml
Storage: Room temperature*

*: Check storage conditions. Products have one year expiration from time of purchase.

Product Description:

2D buffers must solubilize proteins effectively without disturbing the native charge on the proteins. Urea, a common chaotrope, is widely used for solubilization and denaturation of proteins. One of the disadvantages of using urea is carbamylation. Urea in water exists in equilibrium with ammonium cyanate, of whose level increases with increasing temperature and pH. Cyanate reacts with α-amino and ε-amino groups of proteins and induces a change in the isoelectric point. This leads to artifacts in results and therefore carbamylation must be avoided. One way to minimize the risk of carbamylation is to prepare the urea based reagents fresh before each use. Bio Basic Inc. has developed a series of dry urea based pre-mixed and ready-to-use solubilization buffers. Simply add an appropriate volume of the supplied diluent buffer to the dry powder and then use directly to solubilize proteins, saving time and improving the quality of IEF/2D gel electrophoresis. 2D Sample Extraction Buffers are also designed to be used as rehydration buffers for IPG strips. 2D Sample Extraction Buffers are experimentally optimized with proprietary technologies and critical buffering and stabilizing agents, including urea, thiourea, Nonidet® P-40, CHAPS, and sulfobetaines. Our 2D Sample Extraction Buffers are designed to produce optimal protein extraction and significantly improved spot resolution for 2D gel analysis. A range of 2D Sample Extraction Buffers have been developed and depending on the nature of the samples, one or more of the buffers suitable for your applications can be ordered. PL037 is suitable for most applications, however for stronger solubilization effects, we recommend PL038-PL042.

Features:

- Convenient and simple to use extraction buffers.
- Mix and use. Prevents urea induced protein carbamylation.
- Prevents waste of unused reagents. No artifactual protein bands
- Suitable for sample extraction and solubilization for 2D gel electrophoresis and other applications.
- Suitable for rehydration of IPG Strips.

Storage and Transportation:

Transportation at room temperature. Upon receipt, store all of components at 18-25°C.

Compositions:

PL037 Extraction Buffer for 2D Gel with Diluent, 50ml Urea and NP-40
PL038 Extraction Buffer for 2D Gel with Diluent, 50ml Urea and CHAPS
PL039 Extraction Buffer for 2D Gel with Diluent, 50ml Urea, Thiourea & CHAPS
PL040 Extraction Buffer for 2D Gel with Diluent, 50ml Urea, Thiourea, CHAPS & ASB-16
PL041 Extraction Buffer for 2D Gel with Diluent , 50ml Urea, Thiourea, CHAPS & SB 3-10
PL042 Extraction Buffer for 2D Gel with Diluent , 50ml Urea, Thiourea, CHAPS & NDSB 201

T: 1-(800) 313-7224, **W**: www.biobasic.com, **E**: order@biobasic.com, Copyright © Bio Basic Inc. All rights reserved.

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Procedures:

1. Add 1.15 ml Diluent directly into the 1 g PL037, PL038, PL040. Add 1ml Diluent directly into the 1 g PL039, Pl041, PL042. If reconstitution using all components, final volume of 2D Extraction Buffer is 50 ml.
2. If desired, add reducing agents, inhibitors, carrier ampholyte, dye BPB, etc.
3. Mix periodically and incubate at room temperature until you have a clear solution. PL041 might require warming at 25-30°C for a short period (water bath) For any unused buffer, aliquot 2-3 ml each and store at -70°C. Use within 30 days.
4. For each 100 mg of animal tissues, or 0.05 ml (~10 million cells) of wet animal cell pellet, or 50 µl wet insect cell pellet, or 50 mg of E.Coli pellet, use approximately 0.5 ml complete 2D Extraction Buffer.

   NOTE: For plant samples, weigh appropriate amount of plant tissue and cut in to small pieces. Grind the tissue in liquid nitrogen first, then add fresh 0.5 ml complete 2D Extraction Buffer into 100 mg sample and mix well. For yeast samples, digest wall of 50 mg yeast with zymolyase, Snailase, then centrifuge and collect protoplasm. Add 0.5 ml complete 2D extraction Buffer and mix well.

5. Sonicate the suspension at 4°C in an ice cold bath, ensuring no excess heating occurs. Sonication should be performed with bursts for 30-40 seconds and about 5-6 times on ice during Sonication. Care must be taken to prevent heating.
6. Centrifuge the homogenate at 20,000 x g for 30 minutes at 20°C to pellet the cell debris.
7. Use a pipette to transfer the clear extract supernatant into a clean tube without disturbing the pellet.
8. Re-suspend any residual cell debris in 0.05-0.1 ml complete 2D Extraction Buffer. Sonicate and centrifuge as before.
9. Pool supernatants for 2D electrophoresis.

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