# Stabilizor® 2D-GE extraction kit (TISSUE)

## User Manual

## Use with DKT 0002

## 1. Product Description

The Stabilizor 2D-GE extraction kit (Tissue) is designed to ensure efficient homogenization and protein solubilization of heat stabilized tissues. When performing 2D-GE experiments with heat stabilized samples, three aspects should be especially considered:

- Thorough homogenization
- Selection and preparation of the Protein Extraction Buffer components (with or without DTT) following the 2D-GE protocol supplied
- Correct buffer-to-sample ratio to ensure solubilization

## 2. Intended use

The Stabilizor 2D-GE Extraction kit contains all necessary items needed for sample collection and protein extraction prior to 2D-GE. The Protein Extraction Buffer is supplied as three components in order to avoid carbamylation of urea and to prevent oxidation of dithiothreitol (DTT).

The lack of enzymatic activity in heat stabilized samples ensures that samples are not at risk of enzymatic degradation during thawing or sample handling. However, unnecessary freeze-thaw cycles and variations in sample handling should be avoided since non-enzymatic processes may still affect the sample.
**The Stabilizor 2D-GE extraction kit is intended for RESEARCH USE ONLY**

## 3. Materials supplied

- 12 x Maintainor® Tissue
- 1 x Protein Extraction Buffer – Dry Component (26 g)
- 1 x Protein Extraction Buffer – Diluent (25ml)
- 3 x Protein Extraction Buffer – DTT Components (23 mg)
- 36 x Pestles for homogenization
- 36 x Tubes for homogenization
- Quick guide - Stabilizor® 2D-GE extraction kit (Tissue)

## 4. Required (not provided)

- User Manual - Stabilizor® 2D-GE extraction kit (Tissue), available online in PDF format from the Service and Support section at www.denator.com
- Centrifuge capable of spinning 1.5ml tubes at 12 000 x g or more
- Sample storage tubes (recommended: Protein LoBind Tube *(Eppendorf))*
- Protein quantification kit
- Aliquot tubes for Protein Extraction Buffer (>15 ml)
- Dry ice

## 5. Storage & Disposal

- Store kit components prior to use in +4°C to + 8°C
- Store prepared Protein Extraction Buffer below -18°C for up to 3 months.
  *We recommend storing this buffer in 15 ml aliquots*.
- Protein Extraction Buffer containing DTT CANNOT be stored
- Use Protein Extraction Buffer within 12 month from manufacturing date

Follow local regulations when disposing used or unused materials.

# 6. Safety Information

When working with chemicals always work responsibly. Wear suitable protective clothing, disposable gloves and protective goggles.
For more information please consult the appropriate Safety Data Sheets (S.D.S.).
A S.D.S. for each kit component is available online in PDF format from the Service and Support section at www.denator.com.
A S.D.S. is only issued for the individual kit components since the final buffer is not classified as hazardous.

## 6.1 Protein Extraction Buffer, with or without DTT

After preparation of buffer, with or without DTT component, the concentrations of hazardous compounds in the Protein Extraction Buffer are below those set by REACH regulations (1907/2006) as requiring aS.D.S. .
Please consult the S.D.S. for each individual component.

## 6.2 Protein Extraction Buffer - Diluent

Concentrations of hazardous compounds in the Protein Extraction Buffer – Diluent are below the limits for classification of the product as hazardous as set by REACH regulations (1907/2006).

## 6.3 Protein Extraction Buffer – Dry component

**Harmful**

**Dangerous for the environment**

Warning

Possible risk of harm to the unborn child
Limited evidence of a carcinogenic effect
Harmful if swallowed
Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

Safety Measures

Wear suitable protective clothing and gloves
Avoid release to the environment. Refer to special instructions/ Safety data sheets

Note – After dissolution of the Dry component into Diluent, the mixture is not classified as hazardous.

## 6.4 Protein Extraction Buffer – DTT component

**Warning**

Warning

Harmful if swallowed.
Causes skin irritation.
Causes serious eye irritation.
May cause respiratory irritation.

Safety Measures

Avoid breathing dust.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Note – After dissolution of the Dry component into Diluent, the mixture is not classified as hazardous.

# 7. Specific protocol

In the following step-by-step protocol, **important passages are in bold font**.

## 7.1 Heat stabilization

*For more information on the use of the Stabilizor® system, please consult the Stabilizor User Manual.*

1. Collect sample and place in Maintainor Tissue card.
2. Heat stabilize immediately following the Stabilizor User Manual instructions. *If preferred, samples can be frozen and heat-stabilized at a later stage.*
3. If necessary, sub-dissect samples after heat stabilization *but before freezing.*
4. *If samples will be analyzed at a later date*, store samples at -80°C.

## 7.2 Preparation of Protein Extraction Buffer

Each kit contains reagents necessary to prepare 45 ml of Protein Extraction Buffer. This buffer can also be used to rehydrate IPG strips for 2D-GE, or to extract proteins for 1D-GE.
Protein Extraction Buffer is prepared by dissolving the Dry component with the Protein Extraction Buffer Diluent. DTT is added to the Protein Extraction Buffer prior to use. DTT reduces disulfide bonds at cysteine residues to completely denature proteins prior to 2D-GE.

1. Mix Dry Buffer Component and Diluent (25ml) in the Dry Buffer Component bottle.
2. Stir constantly until a clear solution is seen. (Dissolution of urea is an endothermic reaction and takes about 3 h in room temperature. If faster re-solubilization is required transfer the solution to a beaker standing in warm water (**max** 37°C) and stir to speed up the procedure.
3. Store Protein Extraction Buffer below -18°C in aliquots (15 ml) for up to 3 months If not used directly.

**Preparing Protein Extraction Buffer with DTT**

***Note: If proteins are to be labeled with CyDye™ Fluor dyes for 2D-DIGE analysis, omit DTT from the Protein Extraction Buffer and add to the sample extract after labeling, as specified by the dye manufacturer.***

1. Add DTT component to the Protein Extraction Buffer immediately prior to use. **Use one DTT component vial with 15 ml of Protein Extraction Buffer.** Reconstitute the DTT component by adding 1 ml of the 15 ml Protein Extraction Buffer to the DTT vial.
2. Aspirate several times.
3. Transfer the Protein Extraction Buffer containing the DTT component back to the Protein Extraction Buffer bottle.

If the entire Protein Extraction Buffer (45 ml) is to be used at the same time, all three (3) DTT component vials should be included in the buffer.
**Protein Extraction Buffer containing DTT cannot be saved for later use.**

*NOTE: Protein Extraction Buffer (including DTT or not) is compatible with DeStreak Rehydration Reagent (GE Healthcare).*

*NOTE: Carrier ampholytes or bromophenol blue dye may be added if necessary.*

## 7.3 Protein extraction

*For easier handling, perform step 1-4 on dry ice if working with frozen samples.*

1. Pre-weigh the supplied homogenization tubes.
2. Place samples in the homogenization tubes. **Avoid collecting samples larger than 50 mg per tube,** because of the risk of sample loss during open-tube homogenization.
3. Weigh the homogenization tubes and calculate sample weight.
   **Note: Use 10 µl of Protein Extraction Buffer per milligram of sample. For samples smaller than 10 mg, use 100 µl of Protein Extraction Buffer.**
4. Crush the sample coarsely using the supplied pestle.
5. Add approximately 30% of the required volume of Protein Extraction Buffer and homogenize for at least 1 minute. Add remaining buffer and continue the homogenization.
6. Homogenize for at least 2 minutes using the pestle; continue even when no traces of coarse material remain.
7. *Additional micro-tip sonication will improve the protein extraction. Important to keep sample temperature below 37℃.*
8. *Mix tube or vortex sample briefly before centrifuge.*
9. Centrifuge the sample at top speed (at least 12 000 x g) for 25 min to sediment cellular debris.
10. Carefully transfer supernatant into a new tube without disturbing the pellet.
11. The sample is now ready for protein quantification (see below) and 2D-GE.

## 7.4 Protein quantification

Measure the concentration of protein using a urea-compatible kit, e.g. 2-D Quant kit (GE Healthcare), or dilute the sample x7 to reach urea concentrations under 1M to use standard protein assays, e.g. BCA Protein Assay kit (Pierce).

## 7.5 Downstream analysis

Subsequent downstream steps are not affected by heat stabilization and conventional 2D-GE protocols can be followed without alterations.

## 8. References

Goodwin, R. J., A. M. Lang, et al. (2010). "Stopping the clock on proteomic degradation by heat treatment at the point of tissue excision." Proteomics 10(9): 1751-61.

Grassl, J., J. A. Westbrook, et al. (2009). "Preserving the yeast proteome from sample degradation." Proteomics 9(20): 4616-26.

Robinson, P., K. Toney, et al. (1995). "Mass spectrometric and biological characterization of guinea-pig corticotrophin." Regul Pept 56(1): 89-97.

Rountree, C. B., C. A. Van Kirk, et al. (2010). "Clinical application for the preservation of phospho-proteins through in-situ tissue stabilization." Proteome Sci 8: 61.

Scholz, B., K. Sköld, et al. (2011). "Impact of temperature dependent sampling procedures in proteomics and peptidomics - a characterization of the liver and pancreas post mortem degradome." Mol Cell Proteomics 10(3): M900229MCP200.

## 9. Technical assistance

For questions about this kit, the use of the Stabilizor® system and working with heat stabilized tissue, visit www.denator.com for latest application details and technical support.

## 10. Contact

**Denator AB**
Arvid Wallgrens Backe 20
SE 413 46 Göteborg
Sweden
Phone: +46 (0)31 412 890
Fax: +46 (0)31 412 840
Mail: inform@denator.com
**www.denator.com**



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