GE Healthcare

PlusOne Sample Grinding Kit

Product Specification Sheet

PlusOne™ Sample Grinding Kit is designed for the grinding of small tissue or cell samples for protein extraction. The kit consists of fifty 1.5 ml microcentrifuge tubes each containing a small quantity of abrasive grinding resin suspended in water. Fifty pestles for sample grinding are also supplied. The tube is centrifuged to pellet the resin and the water is removed. Extraction solution of choice is added to the tube along with the sample to be ground. A disposable grinding pestle is used to grind the sample. Cellular debris and grinding resin are removed by centrifugation.

Warning
For research use only.
Not recommended or intended for diagnosis of disease in humans or animals.
Do not use internally or externally in humans or animals.

Storage

The kit should be stored at room temperature.

Function testing

Each lot of the PlusOne Sample Grinding Kit is tested to ensure that the proper quantity of grinding resin has been delivered to each tube.

Safety warnings and precautions

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Components

Grinding Tubes:
Microcentrifuge tubes containing grinding resin suspended in water

Pestles:
Pestles for sample grinding

Overview

Effective analysis of the proteins in a tissue sample depends on effective sample disruption. Many analysis techniques, including SDS-PAGE and 2-D electrophoresis, are applied to relatively small tissue samples.

PlusOne Sample Grinding Kit provides a simple and straightforward procedure for disrupting tissue samples of 100 mg or less. The kit contains 1.5 ml microcentrifuge tubes each containing a small quantity of abrasive grinding resin suspended in water. The tube is centrifuged to pellet the resin and the water is removed. Extraction solution of choice is added to the tube along with the sample to be ground. A disposable pestle is supplied to grind the sample. Immediately after grinding, cellular debris and grinding resin are removed by 5–10 min of centrifugation.

PlusOne Sample Grinding Kit will effectively grind most animal and plant tissues. Intracellular organelles are also disrupted, resulting in the liberation and extraction of all proteins soluble in the extraction solution.

Protocol

Preliminary preparations

The tissue of interest may be sliced with a scalpel to yield an appropriately sized fragment, or it may be frozen with liquid nitrogen and broken into small fragments in a mortar and pestle. Sample grinding may be simpler if the tissue is minced prior to adding to the grinding tube.

Required but not provided:

- Microcentrifuge capable of spinning tubes at 12 000 × g or more.
- Vortex mixer
- Extraction solution (examples given below)

Procedure

1. Place one or more of the grinding tubes in a microcentrifuge. Centrifuge briefly at maximum speed to pellet the grinding resin. Use a micropipette to remove as much of the liquid as possible from the grinding resin pellet.
2. Add 200–300 µl of extraction solution of choice to the grinding tube. Vortex to resuspend the grinding resin (see "Examples of protein extraction solutions").
3. Add up to 100 mg of solid tissue sample to the tube.
4. Use a pestle to thoroughly grind the sample. This may require up to one minute of grinding. Add additional extraction solution up to 1 ml if desired.
5. Centrifuge the tube to remove resin and cellular debris. Centrifuge for 5–10 min at maximum speed. Carefully transfer the clear supernatant to another tube. The extract is now ready for further cleanup or analysis by SDS-PAGE, 2-D electrophoresis or other means.

Examples of protein extraction solutions

The following solution is useful for extracting proteins to prepare samples for 2-D electrophoresis.

Extraction solution containing 8 M urea for 2-D electrophoresis

(8 M urea, 2% CHAPS, 40 mM DTT, 0.5% Pharmalyte™ IPG Buffer, 2.5 ml)

	final concentration	amount
Urea (FW 60.06)	8 M	1.20 g
CHAPS[1]	2% (w/v)	50 mg
Carrier ampholyte[2] (Pharmalyte™ or IPG Buffer)	0.5% (v/v)[3]	12.5 µl
DTT (FW 154.2)	40 mM	15.4 mg
Distilled or de-ionized water		to 2.5 ml

[1] Other neutral or zwitterionic detergents may be used at concentrations up to 2% (w/v). Examples include Triton X-100, NP-40, octyl glucoside and the alkylamidosulfobetaine detergents ASB-14 and ASB-16.

[2] Use IPG buffer in the pH range corresponding to the pH range of the IEF separation to be performed, or Pharmalyte in a pH range approximating the pH range of the IEF separation to be performed.

[3] Concentrations greater than 0.5% may be used for some applications. See "2-D Electrophoresis Using Immobilized pH Gradients, Principles and Methods" for guidelines.

The following solution is a more strongly solubilizing solution to prepare samples for 2-D electrophoresis.

Extraction solution containing urea and thiourea for 2-D electrophoresis

(7 M urea, 2 M thiourea, 4% CHAPS, 40 mM DTT, 0.5% Pharmalyte or IPG Buffer, 2.5 ml).

	final concentration	amount
Urea (FW 60.06)	7 M	1.05 g
Thiourea (FW 76.12)	2 M	381 mg
CHAPS[1]	4% (w/v)	100 mg
Carrier ampholyte[2] (Pharmalyte or IPG Buffer)	0.5% (v/v)[3]	12.5 µl
DTT (FW 154.2)	40 mM	15.4 mg
Distilled or de-ionized water		to 2.5 ml

[1] Other neutral or zwitterionic detergents may be used at concentrations up to 2% (w/v). Examples include Triton X-100, NP-40, octyl glucoside and the alkylamidosulfobetaine detergents ASB-14 and ASB-16.

[2] Use IPG buffer in the pH range corresponding to the pH range of the IEF separation to be performed, or Pharmalyte in a pH range approximating the pH range of the IEF separation to be performed.

[3] Concentrations greater than 0.5% may be used for some applications. See "2-D Electrophoresis Using Immobilized pH Gradients, Principles and Methods" for guidelines.

The following solution is useful for extracting proteins to prepare samples for SDS-PAGE.

Laemmli sample buffer

(125 mM Tris-Cl pH 6.8, 2% (w/v) SDS, 10% (v/v) Glycerol, 100 mM DTT, 0.01% (w/v) Bromophenol Blue, 10 ml)

	final concentration	amount
Tris-Cl pH 6.8	125 mM	1.25 ml of a 1 M stock solution[1]
SDS	2% (w/v)	200 mg, or 2 ml of a 10% (w/v) stock solution
Glycerol	10% (v/v)	1.15 ml of 87% glycerol
DTT (FW 154.2)	100 mM	154 mg
Bromophenol Blue	0.01% (w/v)	1 mg
Distilled or de-ionized water		to 10 ml

[1] Stock solution (1 M Tris-Cl pH 6.8) is prepared by dissolving 12.11 g of Tris base in 80 ml of distilled or de-ionized water. Bring the pH to 6.8 using HCl and add distilled or de-ionized water to bring the volume to 100 ml.

Following extraction, the sample solution may be treated to remove interfering substances using PlusOne 2-D Clean-Up Kit or PlusOne SDS-PAGE Clean-Up Kit.

Ordering information

Product	Quantity	Code No.
Sample Grinding Kit	50 samples	80-6483-37

Related products	Quantity	Code No.
Tris	500 g	17-1321-01
Urea	500 g	17-1319-01
CHAPS	1 g	17-1314-01
Triton X-100	500 ml	17-1315-01
Dithiothreitol (DTT)	1 g	17-1318-01
Bromophenol Blue	10 g	17-1329-01
2-D Quant Kit	500 assays, 1–50 µl and up to 50 µg	80-6483-56
2-D Clean-Up Kit	50 samples, 1–100 µl	80-6484-51
SDS-PAGE Clean-Up Kit	50 samples, 1–100 µl	80-6484-70
Mini Dialysis Kit	1 kDa cut-off, up to 250 µl	80-6483-75
Mini Dialysis Kit	1 kDa cut-off, up to 2 ml	80-6483-94
Mini Dialysis Kit	8 kDa cut-off, up to 250 µl	80-6484-13
Mini Dialysis Kit	8 kDa cut-off, up to 2 ml	80-6484-32
2-D Protein Extraction Buffer Trial Kit	for 6 × 10 ml	28-9435-22
2-D Protein Extraction Buffer-I	for 50 ml	28-9435-23
2-D Protein Extraction Buffer-II	for 50 ml	28-9435-24
2-D Protein Extraction Buffer-III	for 50 ml	28-9435-25
2-D Protein Extraction Buffer-IV	for 50 ml	28-9435-26
2-D Protein Extraction Buffer-V	for 50 ml	28-9435-27
2-D Protein Extraction Buffer-VI	for 50 ml	28-9435-28
Nuclease Mix	0.5 ml	80-6501-42
Protease Inhibitor Mix	1 ml	80-6501-23

Related literature	Code No.
Handbook: 2-D Electrophoresis Using Immobilized pH Gradients, Principles & Methods.	80-6429-60

Legal

This product is manufactured by G-Biosciences, USA.

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