
MicroRotorTM Lysis Kit (Plant)

Instruction Manual

Catalog #163-2142

For technical support, call your local Bio-Rad office, or
in the US, call 1-800-4BIORAD (1-800-424-6723)



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Section 1

Introduction

MicroRotofor lysis kits provide convenient, efficient methods for the preparation of protein samples for fractionation with the MicroRotofor cell. The MicroRotofor lysis kit (plant) is designed for use with soft plant tissues and plant cell cultures, and employs tissue maceration and/or solubilization into a chaotropic extraction buffer (Vuillard et al. 1995). For added convenience, the extraction buffer is also used as the sample buffer for isoelectric focusing (IEF) either with the MicroRotofor cell or with IPG strips. The kit also includes components of the ReadyPrep™ 2-D cleanup kit, which are used to remove pigments and other interfering molecules from the plant samples.

Section 2

Kit Specifications

Each MicroRotofor lysis kit (plant) provides sufficient reagent to perform at least 10 extractions (from 1 g samples) and to prepare sample for 10 MicroRotofor runs. More than 10 extractions will be possible with the kit if the samples are smaller than 1 g or if the sample is applied onto IPG strips and not prefractionated with the MicroRotofor cell. Using this kit, extraction from 1 g spinach leaf tissue yields ~20 mg total protein.

Each MicroRotor run using 2.5 mg total protein yields ten 150–250 μ l fractions, and the protein distribution among the fractions will vary depending on the sample. For example, using spinach leaf extracts and ampholytes spanning the pH range 3–10, fractions 3–5 typically contain the highest amounts of protein.

Certificates of analysis and MSDS forms are available upon request.

Items Supplied With Kit

Protein solubilization buffer (PSB) (contains urea, thiourea, NDSB 201, and Tris)	25 g
PSB diluent (contains CHAPS and Tris)	30 ml
ReadyPrep 2-D cleanup kit	1 kit
Instruction manual	1

Items Required But Not Provided

- 1.5 ml microcentrifuge tubes
- 14 ml disposable culture tubes with closures (VWR catalog #60818-725)
- Microcentrifuge capable of spinning at 20,000 x g
- Sonicator with probe

- DTT reducing agent (catalog #161-0611) or TBP reducing agent (catalog #163-2101)
- Carrier ampholytes
- RC DC™ protein assay (catalog #500-0121 or 500-0122)
- Glycerol
- ReadyPrep proteomic grade water (catalog #163-2091) or other ultrapure water
- Liquid nitrogen
- Mortar and pestle

Items Recommended But Not Required

- Protease inhibitor (for example, Sigma catalog #P9599)
- ReadyPrep reduction-alkylation kit (catalog #163-2090)
- Bio-Rad spin filters (catalog #700-7254) or equivalent filtration device

Section 3

Storage Conditions

Shipped at ambient temperature. Store kit components as individually marked. Wash reagent 2 (component of the ReadyPrep 2-D cleanup kit) must be stored at -20°C for at least 1 hr prior to use. For convenience, store wash reagent 2 at -20°C . This kit has a warranty period of 1 year from shipment date, assuming all components are stored as indicated on each label.

Component	Store at
Protein solubilization buffer (PSB), 25 g	RT
PSB diluent, 30 ml	4°C
ReadyPrep 2-D cleanup kit	
Precipitation agent 1, 15 ml	RT
Precipitation agent 2, 15 ml	RT
Wash reagent 2, 50 ml	-20°C
Wash 2 additive, 0.25 ml	RT
Wash reagent 1, 2.0 ml	RT

Section 4

Instructions for Use

Preparation of Protein Solubilization Buffer (PSB) Solution

1. Use only freshly rehydrated buffer. Discard any unused buffer.
2. Allow the PSB dry reagent to warm to room temperature before opening the bottle. Shake the PSB dry reagent bottle for 10–15 sec. Weigh an appropriate amount (each gram of dry reagent will prepare approximately 2 ml buffer solution). Use 1 ml of freshly prepared PSB per 300 mg of plant tissue (Table 1).

Table 1. Guideline for PSB preparation.

# Samples (300 mg tissue)	Volume PSB Needed (ml)	PSB Dry Reagent (g)	PSB Diluent (ml)	Approximate Volume PSB Prepared (ml)
1	1	1	1.1	2
2	2	2	2.2	4
3	3	2	2.2	4
4	4	3	3.3	6
5	5	3	3.3	6

3. For each gram of dry reagent, add 1.1 ml of PSB diluent.
4. Vortex periodically and incubate at room temperature until you have a clear solution (2–3 min).

5. Add reducing agents, protease inhibitors and carrier ampholyte as needed (Table 2)..

Table 2. Additions to PSB solution recommended for various applications. Note that though the applications listed often require use of chaotropes and detergents, these agents are already included in the PSB solution.

Component	Protein Extraction	IEF Separation	
		MicroRotorfor Cell	IPG Strip
Carrier ampholyte	NA	2% (w/v)	0.2% (w/v)
DTT* or TBP*	50–100 mM	50–100 mM	50–100 mM
	2–5 mM	2–5 mM	2–5 mM
Protease inhibitor	According to manufacturer	NA	NA
Bromophenol Blue	NA	NA	0.002% (w/v)
Glycerol	NA	10%	NA

*Not needed if reduction-alkylation is performed at step 11.

Sample Processing

6. Weigh out 1 g of plant tissue and freeze with liquid nitrogen.
7. Grind frozen material in liquid nitrogen with a mortar and pestle to a fine powder.

8. Resuspend 1 g of powdered sample in 3 ml of prepared PSB. Vortex briefly, then sonicate ten times for 20 sec each in an ice bath.
9. Centrifuge at 20,000 x g for 30 min at 20°C.
10. Remove any floating debris by filtering the supernatant using spin filters. If using Bio-Rad's spin filters, centrifuge at 12–16,000 x g for 10 min. Collect filtrate.
11. Determine the protein concentration of the extract. This is best done using the *RC DC* protein assay (catalog #500-0121 or 500-0122), which is compatible with the detergents and reducing agents in PSB. If performing the *RC DC* protein assay, wash the sample twice. (Optional: A reduction and alkylation of the sample is recommended at this point. Refer to the ReadyPrep reduction-alkylation kit, catalog #163-2090).

Note: If applying sample onto an IPG strip (with no MicroRotor run to be performed), proceed to step 1, Section 4 of the ReadyPrep 2-D cleanup kit instruction manual. If a MicroRotor step is to be performed, proceed to step 12. At this point the protein extract can be stored at –70°C.

Preparing Extracts for a MicroRotor Run (See Section 6 of MicroRotor manual for suggestions of alternative sample preparation and load conditions.)

12. Prepare fresh PSB solution containing PSB diluent, glycerol, carrier ampholyte, and DTT or TBP (DTT or TBP is not required if a reduction-alkylation step is performed at step 11). See Table 2 for recommendations.
13. One MicroRotorfor run requires ~2.5 mg protein (1 $\mu\text{g}/\mu\text{l}$) in a total volume of 2.5 ml. Using the above prepared solution, prepare 2.5 ml of a 1 $\mu\text{g}/\mu\text{l}$ dilution of the protein extract. These recommendations are based on spinach leaf as a sample source and may vary depending on sample type. Load the entire 2.5 ml sample into the MicroRotorfor chamber. It may be necessary to add extra PSB solution to fill the chamber completely, eliminating any void volumes.
14. Run the MicroRotorfor cell according to the MicroRotorfor instruction manual (typically 1500 Vh at 1 Watt constant). After the run, harvest the fractions and proceed to step 1, Section 4 of the ReadyPrep 2-D cleanup kit instruction manual. This is important for removal of phenolics and high concentrations of carrier ampholytes.

Note: Following fractionation with the MicroRotorfor cell it is recommended to perform an SDS-PAGE analysis profiling all 10 fractions. This will illustrate the protein content of each fraction. See the Appendix for recommendations pertaining to SDS-PAGE analysis of MicroRotorfor fractions.

For subsequent analysis of MicroRotofor fractions by 2-D PAGE, the ampholyte concentration in samples should not exceed 0.2–0.5%. If fractions contain high amounts of protein, dilution prior to loading onto the IPG strip (by 1:10 or greater) will be sufficient to reduce the ampholyte concentration. In cases where protein levels are lower, use of the ReadyPrep 2-D cleanup kit (catalog #163-2130) for ampholyte removal is recommended.

Section 5

Appendix

Preparation for SDS-PAGE

CHAPS, a component of the PSB diluent, may interfere with SDS-PAGE. Remove CHAPS from the extracts (for example, with the ReadyPrep 2-D cleanup kit) or dilute the extracts 1:1 with 1x Laemmli buffer prior to SDS-PAGE.

Preparation for IEF on an IPG Strip

The sample extract can be loaded directly onto an IPG strip after appropriate dilution. See Table 3 for recommendations on how much sample to load onto an IPG strip. Dilution of the sample can be done using the protein solubilization buffer (PSB) as a rehydration/sample buffer. However, some critical components need to be added to the PSB solution to make it IEF-compatible (Table 2).

Table 3. Recommended protein loads for IPG strips.

	IPG Strip Length				
	7 cm	11 cm	17 cm	18 cm	24 cm
Rehydration volume/strip	125 μ l	185 μ l	300 μ l	315 μ l	410 μ l
Protein load					
Silver stain	5–20 μ g	20–50 μ g	50–80 μ g	50–80 μ g	80–150 μ g
Coomassie G-250	50–100 μ g	100–200 μ g	200–400 μ g	200–400 μ g	400–800 μ g
Flamingo™, SYPRO Ruby	2.5–75 μ g	10–150 μ g	25–300 μ g	25–300 μ g	40–600 μ g

The suggestions made in Table 3 are a general rule of thumb. Increased protein loads may be required for micro-range IPG strips and for samples of higher protein complexity.

Section 6

Reference

Vuillard L et al., Non-detergent sulfobetaines: a new class of mild solubilization agents for protein purification, *Biochem J* 305, 337–343 (1995)

Section 7

Product Information

Catalog #	Description
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Sample Preparation Kits

163-2141	MicroRotofor Lysis Kit (Mammal)
163-2142	MicroRotofor Lysis Kit (Plant)
163-2143	MicroRotofor Lysis Kit (Yeast)
163-2144	MicroRotofor Lysis Kit (Bacteria)
163-2145	Protein Solubilization Buffer (PSB)
163-2146	ReadyPrep Mini Grinders, 20 tubes with resin and pestles
163-2130	ReadyPrep 2-D Cleanup Kit, 50 preps
163-2140	ReadyPrep 2-D Cleanup Kit, 5 preps
170-2836	MicroRotofor Syringes, 3 ml and 10 ml, 3 each

Protein Quantitation Kits (see also bulletin 2610)

500-0121	<i>RC DC</i> Protein Assay Kit I, 500 standard assays, bovine γ -globulin standard
500-0122	<i>RC DC</i> Protein Assay Kit II, 500 standard assays, bovine serum albumin standard

Buffer Components

161-0611	Dithiothreitol (DTT), 5 g
163-2101	Tributylphosphine (TBP), 200 mM, 0.6 ml
163-2091	ReadyPrep Proteomic Grade Water, 500 ml
163-2094	Bio-Lyte® 3/10 Ampholyte, 100x, 1 ml
161-0737	Laemmli Sample Buffer, 1x, 30 ml

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