MicroRotofor™ Lysis Kit (Mammal)

Instruction Manual

Catalog #163-2141

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, effective methods for the preparation of protein samples for fractionation with the MicroRotofor cell. The MicroRotofor lysis kit (mammal) is designed for use with mammlian tissues and 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) with the MicroRotofor cell or with IPG strips. Though the kit is intended for use with mammalian tissues, it may be applied to other animal tissues and to prepare samples for purposes other than IEF with the MicroRotofor cell, such as IEF with IPG strips, 2-D analysis, or SDS-PAGE.

Section 2
Kit Specifications

Each MicroRotofor lysis kit (mammal) provides sufficient reagent to perform at least 15 extractions (from 100 mg tissue samples) and to prepare samples for 15 MicroRotofor runs. More than 15 extractions will be possible if samples are smaller than 100 mg or if the sample is applied onto IPG strips (and not prefractionated with the MicroRotofor

cell). Expected protein recovery using this kit is approximately 20% (based on studies using 100 mg rat brain tissue, mouse liver tissue, and Jurkat cells), and the extraction process requires approximately 1 hr.

Each MicroRotofor run using 2.5 mg total protein yields ten 150–250 µl fractions, and the protein distribution among the fractions will vary depending on the protein sample. For example, using rat brain samples and ampholytes spanning the pH range 3–10, fractions 3–7 typically contain the most protein. Fractions are cleaned up using the ReadyPrep™ 2-D cleanup kit, then are ready for downstream applications (Harbers et al. 2005). As a starting point, 50 µl of a fraction is generally enough to run triplicate 2-D gels using 11 cm IPG strips and stained with Flamingo™ fluorescent stain (catalog #161-0490) for visualization.

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, Tris)	30 ml
ReadyPrep mini grinders	2 packets of 10
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Items Required But Not Provided

- 1.5 ml microcentrifuge tubes
- Microcentrifuge capable of spinning at 20,000 x g
- 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

Items Recommended But Not Required

- Protease inhibitor (for example, Sigma catalog #P8340)
- ReadyPrep reduction-alkylation kit (catalog #163-2090)
- ReadyPrep 2-D cleanup kit (catalog #163-2130)

Section 3
Storage Conditions

Shipped at ambient temperature. Store kit components as individually marked. 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 mini grinders	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 PSB per 100 mg of tissue or 50 µl of wet cell pellet (Table 1).

Table 1. Guideline for PSB preparation.

# Samples (100 mg tissue or 50 µl wet cell pellet)	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	
		MicroRotofor Cell	IPG Strip
Carrier ampholyte	NA	2% (w/v)	0.2% (w/v)
DTT* or TBP*	50–100 mM 2–5 mM	50–100 mM 2–5 mM	50–100 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 13.

Sample Processing**

6. If using ReadyPrep Mini grinders, centrifuge the tubes containing resin in a microcentrifuge at 20,000 x g for 20 sec and remove the collected liquid.
7. Weigh out tissue sample (up to 100 mg per ReadyPrep mini grinder), and add it to the tube containing resin.
8. Add 500 µl of prepared PSB solution to the tube containing resin.
9. Grind the sample using the supplied matching pestle. Once larger tissue pieces have been reduced, add an additional 500 µl of prepared PSB solution. Grinding times may vary for different tissue samples, but typically 5 min of grinding should be sufficient.

Note: Standardizing the grinding procedure helps ensure reproducible results (for example, record the length of grinding time or count the number of pestle turns).

10. Centrifuge the tubes at 20,000 x g for 30 min at 20°C to pellet the resin and cellular debris.

** For cultured cells, it may not be necessary to use the ReadyPrep mini grinders since cell lysis will be achieved by vortexing with PSB for 2 min. Cultured cell starting material should be an approximately 50 µl wet cell pellet. As an example using Jurkat cells, this will be roughly 15 x 10^6 cells. After steps 1 through 5, begin sample processing at step 10.

11. Transfer the supernatant carefully into a new tube without disturbing the pellet.

12. Resuspend the residual cell pellet in 250 µl of prepared PSB, and repeat steps 9 and 10. Collect the supernatant and pool with the first supernatant.

13. 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 present in PSB. If performing the *RC DC* protein assay, keep in mind that two washes of the sample are recommended. (Optional: A reduction and alkylation of the sample is recommended at this point in the procedure. Refer to the ReadyPrep reduction-alkylation kit, catalog #163-2090). Store the protein extract at –70°C, apply it directly onto an IPG strip (see Appendix for details), or proceed to step 14.

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

14. 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 13). One MicroRotofor run requires 2.5 ml sample. See Table 2 for recommendations.

15. One MicroRotofor requires ~2.5 mg protein (1 µg/µl) in a total volume of 2.5 ml. Using the above prepared solution, prepare 2.5 ml of a 1 µg/µl dilution of the protein extract. These recommendations are based on rat brain as a sample source and may vary depending on sample type. Load the entire 2.5 ml sample into the MicroRotofor chamber. It may be necessary to add extra PSB solution to fill the chamber completely, eliminating any void volumes.

16. Run the MicroRotofor cell according to MicroRotofor manual instructions — typically 1500 Vhs at 1 Watt (constant) when using PSB solution as recommended in this kit.

Note: Following fractionation with the MicroRotofor 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 MicroRotofor 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) 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

Following step 13, the sample extract can be loaded directly onto an IPG strip after appropriate dilution. See Table 3 for recommendations on how much protein sample to load onto an IPG strip. Dilution of the sample can be done using protein solubilization buffer (PSB) as a rehydration/sample buffer. However, some critical components must 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 µl	185 µl	300 µl	315 µl	410 µl
Protein load					
Silver stain	5–20 µg	20–50 µg	50–80 µg	50–80 µg	80–150 µg
Coomassie G-250	50–100 µg	100–200 µg	200–400 µg	200–400 µg	400–800 µg
Flamingo™, SYPRO Ruby	2.5–75 µg	10–150 µg	25–300 µg	25–300 µg	40–600 µg

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 References

Harbers A et al., Fractionation by liquid-phase isoelectric focusing in the MicroRotofor cell: improved detection of low-abundance proteins, Bio-Rad bulletin 5344 (2005)

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

Section 7
Product Information

Catalog #	Description
Sample Preparation Kits (see also bulletin 2934)	
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
163-2090	ReadyPrep Reduction-Alkylation Kit, 100 preps
170-2836	MicroRotofor Syringes, 3 ml and 10 ml, 3 each
Protein Quantitation Kits (see also bulletin 2610)	
500-0121	*RC DC* Protein Assay Kit I, 500 standard assays, bovine γ-globulin standard
500-0122	*RC DC* 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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