MicroRotofor™ Lysis Kit (Bacteria)

Instruction Manual

Catalog #163-2144

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 (bacteria) is designed for use with both gram negative and gram positive bacterial cultures, and employs enzymatic digestion of the cell wall (Repaske et al. 1956) followed by 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.

Section 2
Kit Specifications

Each kit provides sufficient reagent to prepare 15 extractions for loading onto the MicroRotofor. This is based on each lysis being performed on 4.5 ml fresh *E. coli* culture (OD_{600} = 1.455), which should yield a wet cell pellet of roughly 50 µl. More than 15 extractions will be possible if the sample is applied onto IPG strips (and not prefractionated with the MicroRotor cell).

This kit can be used with both gram negative and gram positive bacteria. 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 sample. For example, using *E. coli* extracts and ampholytes spanning the pH range 3–10, fractions 3–6 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
Bacteria suspension buffer (contains sodium phosphate, sodium chloride, potassium chloride, potassium phosphate)	25 ml
Lysozyme enzyme, 1,500 units/µl, prepared from chicken egg, MW = 14.3 kD, pI = 9.32 (Swiss-Prot/TrEMBL acession number LYSC-CHICK P00698)	1.0 ml
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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)
- ReadyPrep™ proteomic grade water (catalog #163-2091) or other ultrapure water
- 37°C heat block or water bath
- Sonicator with probe

Items Recommended But Not Required

- Protease inhibitor (for example, Sigma catalog #P8465)
- 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. Note: Lysozyme is shipped at room temperature but should be stored at –20°C upon receipt. 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
Bacteria suspension buffer, 25 ml	RT
Lysozyme, 1,500 U/µl, 1 ml	-20°C

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 50 µl of wet cell pellet (Table 1).

Table 1. Guideline for PSB preparation.

# Samples (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*	50–100 mM	50–100 mM	50–100 mM
or			
TBP*	2–5 mM	2–5 mM	2–5 mM
Protease inhibitor	According to manufacturer	NA	NA
Bromophenol Blue	NA	0.002% (w/v)	NA
Glycerol	NA	10%	NA

*Not needed if reduction-alkylation is performed at step 17.

6. Suspend the wet bacterial cell pellet (~50 µl) in 500 µl of bacteria suspension buffer.

Note: For best results, use a wet cell pellet from a freshly grown bacterial culture. Do not use frozen or stored stocks.

7. Gently flick the bottom of the tube to resuspend the cell pellet (no longer than 1 min). If the cell pellet doesn't resuspend readily, the cells may no longer be viable for spheroplast preparation. Use fresh cultures.
8. Mix the lysozyme by gently flicking the vial and add it to the sample (5 µl of lysozyme per 50 µl of wet cell pellet). Mix gently.
9. Incubate the suspension at 37°C for 60 min.
10. Centrifuge the suspension at 5,000 x g for 10 min. Remove and discard the supernatant carefully, leaving the spheroplast pellet in the tube.

Note: If the majority of the sample is mucous-like and difficult to pipet, the spheroplasts may have lysed. Reduce the incubation time or start with a fresh culture.

11. Optional wash step: Add 500 µl of bacteria suspension buffer to the spheroplast pellet. Resuspend the spheroplasts by gently tapping the tube. Centrifuge again as above and discard the supernatant.

Note: This step washes away the lysozyme from the protein sample. Including this wash step may slightly compromise the bacterial protein yield since it may remove any bacterial proteins existing outside the spheroplast wall

and/or further lyse some of the spheroplasts. Should you choose to eliminate this step, you may anticipate the migration of lysozyme on a 2-D gel by knowing its pI (9.32) and molecular weight (14.3 kD).

12. Add 500 µl of freshly prepared PSB solution to the spheroplast pellet.
13. Sonicate the suspension with an ultrasonic probe to break down the cell membrane and genomic DNA. Sonication should be performed in an ice bath to prevent heating. Sonication should be performed with bursts of 10–15 sec, with chilling of the suspension on ice between ultrasonic bursts.
14. Centrifuge at 20,000 x g for 30 min at 20°C and collect the clear lysate.
15. Resuspend the residual cell debris in 250 µl of PSB solution. Sonicate the suspension once briefly. Repeat the centrifugation in step 14, collect the supernatant, and pool with the first supernatant.
16. 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.

17. 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.

18. Store the protein extract at –70°C, apply it directly onto an IPG strip (see Appendix for details), or proceed to step 19.

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

19. Prepare fresh PSB solution containing PSB diluent, carrier ampholyte and DTT or TBP (DTT or TBP is not required if a reduction-alkylation step is performed at step 17). See Table 2 for recommendations.

20. One MicroRotofor run 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. 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.

21. Run the MicroRotofor cell according to the MicroRotofor instruction manual.

Note: Following fractionation with the MicroRotofor, 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) 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

Following step 17, the sample extract can be loaded 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 need to be added to the PSB solution to make it IPG-compatible (Table 2).

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.

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

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)

Repaske R et al., Lysis of gram-negative bacteria by lysozyme, Biochem Biophys Acta 22, 189–191(1956)

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	
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

Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Dr.
Hercules, CA 94547 USA
(510) 741-1000
1-800-424-6723

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