MicroRotofor™ Lysis Kit (Yeast)

Instruction Manual

Catalog #163-2143

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 (yeast) is designed for use with yeast cultures, and employs enzymatic digestion of the cell wall (Scott et al. 1980) 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 MicroRotofor lysis kit (yeast) provides sufficient reagent to perform at least 15 extractions (from 5 ml yeast culture with OD_{600} = 1.48, which should yield a 60 µl wet cell pellet) and to prepare sample for 15 MicroRotofor runs. More than 15 extractions will be possible with the kit if the sample is applied onto IPG strips (and not prefractionated with the MicroRotofor cell).

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 *Saccharomyces cerevisiae* and ampholytes spanning the pH range 3–10, fractions 2–4 typically contain the most protein.

Certificates of analysis and MSDS forms are available upon request.

Items Supplied With the Kit

Protein solubilization buffer (PSB) (contains urea, thiourea, NDSB 201, and Tris)	25 g
PSB diluent (contains CHAPS and Tris)	30 ml
Yeast suspension buffer (contains sodium phosphate, sodium chloride, potassium chloride, and potassium phosphate)	15 ml
Lyticase enzyme, 5 units/µl, prepared from *Arthrobacter luteus*, MW = 54.6 kD, pI = 6.33 Swiss-Prot/TrEMBL accession number E13B-ARTSW (Q59146). See Appendix for more information	2 x 0.5 ml
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Items Required But Not Provided

- 1.5 ml microcentrifuge tubes
- 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
- β-mercaptoethanol

Items Recommended But Not Required

- Protease inhibitor (for example, Sigma catalog #P8215)
- 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: Lyticase 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
Yeast suspension buffer, 15 ml	RT
Lyticase, 1.5 U/µl, 2 x 0.5 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 60 µl of wet cell pellet (Table 1).

Table 1. Guideline for PSB preparation.

# Samples (60 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 17.

Sample Processing

6. Suspend the wet yeast cell pellet (~60 µl) in 100 µl of yeast suspension buffer.

Note: For best results, use a wet cell pellet from a freshly grown yeast culture.

7. Add 1 µl of β-mercaptoethanol per 100 µl of yeast suspension buffer.
8. Vortex until the cell suspension becomes homogenous.
9. Flick the vial of lyticase enzyme to mix. Add 10 µl of lyticase enzyme per 100 µl of yeast cell pellet. Gently mix. Lyticase will hydrolyze poly-(beta-1,3-glucose) for lysis of the yeast cell wall.
10. Incubate the cell suspension at 37°C for 30–60 min.
11. Centrifuge the suspension at 10,000 x g for 5 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.

12. Optional wash step: Add 600 µl of yeast 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 lyticase from the protein sample. Including this wash step may compromise the yeast protein yield. Should you choose to eliminate this step, you may anticipate the migration of lyticase on a 2-D gel by knowing its pI (6.33) and molecular weight (54.6 kD).

13. Add 600 µl of freshly prepared PSB solution to the spheroplast pellet.
14. Sonicate the suspension to break down the cell membrane and the genomic DNA. Sonication should be performed in an ice bath to prevent heating. Sonication should be performed with bursts of 20–30 sec, with chilling of the suspension on ice between bursts.
15. Centrifuge at 20,000 x g for 30 min at 20°C and collect the clear lysate.
16. Resuspend the residual cell debris in 250 µl of PSB solution. Sonicate the suspension once briefly. Repeat the centrifugation in step 15, collect the supernatant, and pool with the first supernatant.
17. 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, 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 directly onto an IPG strip (see Appendix for recommendations), or proceed to step 18.

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

18. 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 17). See Table 2 for recommendations.

19. One MicroRotofor run requires ~2.5 mg protein (1 µg/µl) in a total volume of 2.5 ml. Using the above prepared PSB 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.

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

Note: Following fractionaction 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) 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 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.

Lyticase Enzyme

This enzyme is prepared from *Arthrobacter luteus*. The primary yeast lytic activity is beta1, 3-glucan laminaripentaohydrolase, which hydrolyzes glucose polymers at the beta-1,3-glucan linkages, releasing laminaripentaose as the principal product.

Unit definition: One lytic unit is defined as a 10% decrease in absorbance at A_{800} in 30 min.
Assay condition: 50 mM potassium phosphate, pH 7.5, 10 mM β-mercaptoethanol in 1 ml yeast cell suspension of A_{800} 0.8 to 1.0.

Store at –20°C when frequently used. Store below –70°C for infrequent usage (less than once a month). Lyticase is stable for 1 year at –20°C and for many years below –70°C.

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)

Scott J et al., Lyticase: Endoglucosnase and protease activities that act together in yeast cell lysis, J Bacteriol 142, 414–423 (1980)

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

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

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