

Protein Isolation & Quantification from Human Lymphoblastoid Cell Line

Note: This protocol assumes the investigator is beginning with one T75 Cell Culture Flask containing human lymphoblastoid cells grown to 40-50% confluence prepared by the GMB using GMB013 Lymphoblastoid Care Protocol.

Protein Isolation from Human Lymphoblastoid cells

- 1) Prepare sufficient '**complete**' M-PER lysis buffer. (see below)
- 2) Transfer contents of T75 flask (media + cells) to 50 ml polypropylene conical centrifuge tube.
- 3) Pellet the cells at 2000 rpm for 10 minutes.
- 4) Aspirate off supernatant.
- 5) Resuspend pellet in 500 µl '**complete**' M-PER.
- 6) Transfer to flip-top microcentrifuge tube.
- 7) Vortex for 10 seconds.
- 8) Spin @ 13200 (max) rpm @ 4°C for 10 minutes.
- 9) Carefully aliquot supernatant:
 - a. 10 µl for protein assay → Store at room temperature until assayed.
 - b. 100 µl for analysis → Freeze @ -80°C. (Clear 1.5 ml seal-top tube.)
 - c. 400 µl for stock → Freeze @ -80°C. (Purple 1.5 ml seal-top tube.)
- 10) Aliquot 10 µl '**complete**' M-PER for protein assay control.

'Complete' M-PER Protein Lysis Buffer

# of Samples			1-9	10-18	19+
	[Stock]	[Final]	Volume	Volume	Volume
Leupeptin	5 mg/ml	5 µg/ml	5 µl	10 µl	15 µl
Pepstatin	1 mg/ml	5 µg/ml	25 µl	50 µl	75 µl
Aprotinin	5 mg/ml	5 µg/ml	5 µl	10 µl	15 µl
PMSF	1 M	500 µM	2.5 µl	5 µl	7.5 µl
NaOV	1 M	2 mM	10 µl	20 µl	30 µl
NaF	1 M	50 mM	250 µl	500 µl	750 µl
β-glycerophosphate	1 M	10 mM	50 µl	100 µl	150 µl
M-PER Buffer			<u>4.75 ml</u>	<u>9.50 ml</u>	<u>14.25 ml</u>
Total Volume			5 ml	10 ml	15 ml

GMB005

Inhibitor Preparation

****Whenever possible, recommend purchasing amounts of inhibitor that can be prepared in their entirety – to avoid error in weighing/transferring small masses of solids. Adjust volume of solvent as necessary.****

Pepstatin:	1 mg/ml	Dissolve 5 mg pepstatin in 5 ml acidified EtOH (0.5 ml Acetic Acid in 4.5 ml EtOH) (Recommend 500 or 1000 µl aliquots)
Aprotinin:	5 mg/ml	Dissolve 5 mg aprotinin in 1 ml dH ₂ O (Recommend 100 or 200 µl aliquots)
Leupeptin:	5 mg/ml	Dissolve 5 mg leupeptin in 1 ml dH ₂ O (Recommend 100 or 200 µl aliquots)
PMSF:	1 M	Dissolve 0.34 g in 2 ml DMSO (TOXIC – wear mask when measuring out PMSF) (Recommend 100 µl aliquots)
NaOV:	1 M	Dissolve 0.72 g in 2 ml boiling dH₂O (must be hot and may need to be acidified) (Recommend 200 µl aliquots)
NaF:	1 M	Dissolve 0.419 g in 10 ml dH ₂ O (Recommend 1000 or 1500 µl aliquots)
β-glycerophosphate:	1 M	1.08 g in 5 ml dH ₂ O (Recommend 1000 µl aliquots)

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BCA Protein Assay

- 1) Dilute 10 µl aliquot of protein lysate (optimize for estimated yield):
 - a. with 30 µl dH₂O (1:4 dilution)
 - b. with 70 µl dH₂O (1:8 dilution) [Recommended]**
 - c. with 90 µl dH₂O (1:10 dilution)
- 2) Transfer three 10 µl aliquots of each protein standard into a 96-well optical plate.
- 3) Transfer three 10 µl aliquots of each diluted unknown to optical plate.
- 4) Prepare sufficient amount of BCA reagent (**see table below**).
- 5) Transfer 200 µl BCA reagent into each well of the optical plate.
- 6) Incubate 30 minutes @ 37°C OR 2 hours @ 25°C.
- 7) Spec @ 562 nm. (w/ plate reader – preset program available.)

Protein Standard Curve (Albumin):*Albumin Stock: 2 mg/ml*

[Standard] (mg/ml)	V(Stock) (µl)		V(dH ₂ O) (µl)	V(Final) (µl)
1.50	600	+	200	800 µl
1.25	500	+	300	800 µl
1.00	400	+	400	800 µl
0.75	300	+	500	800 µl
0.50	200	+	600	800 µl
0.25	100	+	700	800 µl
0.10	40	+	760	800 µl
0.00	0	+	800	800 µl

BCA Reagent (50:1 A:B)

Reagent A	Reagent B	# of Assays
25 ml	500 µl	96+ (1 plate / 8 standards & 24 unknowns)