

# PROTOCOL: P100 Lysis Buffer Preparation

## Purpose

To prepare the cellular lysis buffer for the P100 assay

## Preparation

1. Begin thawing all stock aliquots of additives {BUF\_M05 through BUF\_M10} on ice

## Materials

- Urea, Sigma-Aldrich, Cat. No. U0632-1K {BUF\_M01}
- 1M NaCl solution in water, Sigma-Aldrich, Cat. No. 71376-1K {BUF\_M02}
- 1M Tris pH 8.0 solution in water, Invitrogen, Cat. No. 15568-025 {BUF\_M03}
- 500 mM EDTA solution in water, Sigma-Aldrich, Cat. No. E7889-100ML {BUF\_M04}
- 1 mg/mL aprotinin solution in water, Sigma-Aldrich, Cat. No. A6103-1MG {BUF\_M05}
- 2 mg/mL leupeptin solution in water, Roche, Cat. No. 11017101001 {BUF\_M06}
- 1M NaF solution in water, Sigma-Aldrich, Cat. No. S7920-100G {BUF\_M07}
- PIC3 (Phosphatase Inhibitor Cocktail 3), Sigma-Aldrich, Cat. No. P0044 {BUF\_M08}
- PIC2 (Phosphatase Inhibitor Cocktail 2), Sigma-Aldrich, Cat. No. P5726 {BUF\_M09}
- 100 mM PMSF solution in ethanol, Sigma-Aldrich, Cat. No. 93482-50ML-F {BUF\_M10}
- HPLC-grade water, JT Baker, Cat. No. 4218-03 {BUF\_M11}

## Assets

- 100 mL flask {BUF\_A01}

## Reagent Mixes

ID	Name	Step	Composition	Stock Volume	Notes
BUF_M02	1M NaCl	BUF	5.844 g per 100 mL of water	100 mL	Store at room temp
BUF_M03	1M Tris pH 8.0	BUF	121.14 g per 1 L of water	1 L	Adjust to pH 8.0 with HCl or use pre-made from Sigma, store at room temp
BUF_M05	1 mg/mL aprotinin	BUF	1 mg per 1 mL of water	1 mL	Store in 150 uL aliquots at 4 C

BUF_M06	2 mg/mL leupeptin	BUF	5 mg per 2.5 mL of water	2.5 mL	Store in 360 uL aliquots at -20 C
BUF_M07	1M NaF	BUF	419.9 mg per 10 mL of water	10 mL	Store in 710 uL aliquots at 4 C
BUF_M08	PIC3	BUF	As is from vial; aliquot	5 mL	Store in 710uL aliquots at 4C
BUF_M09	PIC2	BUF	As is from vial; aliquot	5 mL	Store in 710uL aliquots at 4C
BUF_M10	100 mM PMSF	BUF	As if from vial; aliquot	50 mL	Store in 710 uL aliquots at -20 C

## Procedure

*NOTE: Procedure calculations reflect a final volume of 100 mL of urea solution and 70 mL of lysis buffer*

1. Weigh out 48 grams of urea {BUF\_M01} and place in 100 mL flask {BUF\_A01}
2. Add 7.5 mL of 1M NaCl {BUF\_M02}, 5 mL of 1M Tris pH 8.0 {BUF\_M03} and 200 uL of 500 mM EDTA {BUF\_M04} to the urea
3. Add approximately 30 mL of HPLC-grade water {BUF\_M11} to the flask and mix until the urea is dissolved
  - If the urea will not fully dissolve, add water {BUF\_M11} in 5 mL amounts until all the urea is in solution

*NOTE: Flask will get cold, warm with hands or by keeping on bench at room temperature. Swirl solution occasionally to facilitate dissolution. **All urea must be dissolved before proceeding to Step 4***

4. Bring total volume up to 100 mL with HPLC-grade water {BUF\_M11}
5. Remove 66.71 mL of urea solution and place in new, labeled 100 mL flask {BUF\_A01}

*NOTE: Save remaining urea solution to be used later*
6. Immediately before use add the following reagents to the 66.71 mL of urea solution in the following volumes:
  - 1 mg/mL aprotinin: 140 uL {BUF\_M05}
  - 2 mg/mL leupeptin: 350 uL {BUF\_M06}
  - 1M NaF: 700 uL {BUF\_M07}
  - PIC3: 700 uL {BUF\_M08}
  - PIC2: 700 uL {BUF\_M09}
  - 100 mM PMSF: 700 uL {BUF\_M10}
7. Mix thoroughly and keep on ice until use

NOTE:

Reagent	Material Code	Unit	Amounts for 100mL Lysis Buffer Base	Final Concentration	Location
Urea	BUF_M01	g	48	8M	Solid Chemicals
1M NaCl	BUF_M02	uL	7500	75mM	Robot Room Shelf
1M Tris pH 8.0	BUF_M03	uL	5000	50mM	4°C
500mM EDTA	BUF_M04	uL	200	1mM	Robot Room Shelf
<b>Add to 66.71 mL of urea solution:</b>					
1mg/ml Aprotinin in H2O	BUF_M05	uL	140	2 µg/ml	4°C
2mg/ml Leupeptin in H2O	BUF_M06	uL	350	10 µg/ml	-20°C
1M NaF in H2O	BUF_M07	uL	700	10mM	4°C
PIC3 (Phosphatase inhibitor cocktail 3)	BUF_M08	uL	700	1:100 dilution	4°C
PIC2 (Phosphatase inhibitor cocktail 2)	BUF_M09	uL	700	1:100 dilution	4°C
100mM PMSF in EtOH- add right before use	BUF_10	uL	700	1mM	-20°C