Covaris
the pre-analytical advantage

PART NUMBER
520099

Protein Extraction Buffer N

Designed for native applications preserving protein conformation and biological activity

INTRODUCTION

The Covaris **Protein Extraction Buffer N** (PN 520099) is a physiological buffer optimized for the extraction of proteins in their native conformation. The reagent does not use polyethylene oxide series detergents such as Triton X-100 for protein extraction, since the detergent has a micellar molecular weight of 80,000 Daltons and is difficult to remove by dialysis. Native proteins extracted in the Protein Extraction Buffer N are directly compatible with immunoassay, protein assay, and Blue Native PAGE. Since the reagent does not diminish protein binding, it can be used directly with most affinity enrichment schemes.

KIT CONTENTS

- Bottle N.....Protein Extraction Buffer N, 18 mL
- Vial H.....100X Protease Inhibitor Cocktail. 0.25 mL (optional)
- EDTA.....100X EDTA, 0.25 mL (optional)

STORAGE

Kit components can be stored at 4°C for one year.

PREPARATION OF KIT REAGENTS

1. Bottle N is ready to use. Do not dilute prior to use.
2. When applicable, add Protease Inhibitor Cocktail (Vial H) to the prepared Buffer N immediately before use. **NOTE: Some protease inhibitors are irreversible and may have deleterious effects on biological activity. Add protease inhibitors when it is determined they will not interfere with downstream analysis.**
3. When applicable, add EDTA (Vial E) to the prepared Buffer N. **NOTE: EDTA reversibly inactivates enzymes by the chelation of metal ion cofactors. EDTA has been shown to denature some proteins.**

PROTEIN EXTRACTION PROTOCOL

The below protocol is abbreviated. For detailed protein extraction protocols with cryoPREP Extraction Systems, including the t-PREP, please refer to http://covarisinc.com/resources/protocols/.

1. Add the appropriate volume of Native Protein Extraction Buffer N to cells or cryofractured tissue in the respective AFA Tube or t-PREP for homogenization in a Focused-ultrasonicator.
2. Process samples in a Covaris Focused-ultrasonicator according to the conditions listed in Table 1. A time course should be conducted to determine the optimum conditions for each tissue type and protein of interest.
3. Following AFA, transfer the sample to a 1.5 mL microcentrifuge tube and centrifuge at maximum speed to pellet cellular debris. Transfer the supernatant to a new 1.5 mL microcentrifuge tube for analysis.
4. Buffer N is compatible with the Bradford protein assay and other similar protein assays to determine the protein concentration. Buffer N should be used as the assay blank and for serial dilution of protein standard.

	TC12x12	t-PREP
Volume	1 mL	250 mL
Tissue Mass	< 200 mg	< 10 mg
Duty Cycle	10%	5%
Peak Incident Power (S220 and E220) Intensity (S2 and E210)	70 Watts Level 2	35 Watts Level 1
Cycles per burst	200	
Processing Time	Empirical	
Bath Temperature	7°C	
Power Mode (S2 and E210)	Frequency sweeping	
Degas Mode	Continuous	
Water level (RUN)* S2/S220 E210/E220	 Level 8 Level 5	 Level 5 NA

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