PROTOCOL

truXTRAC™ Protein Extraction Buffer N

Adaptive Focused Acoustics™ (AFA) - enhanced reagent for Native applications preserving protein conformation and biological activity

Product PN 520099

Contents

Intended Use

truXTRAC product is not intended for the diagnosis, prevention, or treatment of a disease.

Introduction

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.

Revision History

Part Number	Revision	Date	Description of change
010389	A	4/17	As released

Kit Contents

Bottle N	Protein Extraction Buffer N, 18 mL
Vial H (optional)	0.25 mL of 100X Protease Inhibitor cocktail in DMSO
Vial E (optional)	0.25 mL of 100X EDTA

SDS information is available at http://covarisinc.com/resources/safety-data-sheets/

Storage

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

Preparation Of Kit Reagents

1. Buffer N is ready to use. Do not dilute prior to use.

2. When applicable, add Protease Inhibitor Cocktail (Vial H) to Buffer N immediately before use.

NOTE: Some protease inhibitors are irreversible and may have deleterious effect 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 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 -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 an appropriate size microcentrifuge tube and centrifuge at maximum speed to pellet cellular debris. Transfer the supernatant to a new microcentrifuge tube for analysis.

4. Buffer N is compatible with Bradford and similar protein assays to determine the protein concentration. Buffer N should be used as the assay blank and for serial dilution of protein standard.

Table 1

	milliTUBE	**t-PREP**	**microTUBE**
Volume	1 mL	250 µL	130 µL
Tissue Mass	< 200 mg	1- 10 mg	10-60 mg
Cell Number	10^8-10^9	NA	10^5-10^7
Peak Incident Power (S220 and E220)	25 -75 Watts	25 – 50 Watts	25 -50 Watts
Peak Incident Power (M220)	50 -75 Watts	NA	35 -70 Watts
Duty Factor	10%	5%	10%
Cycles per burst	200	200	200
Processing Time	Empirical	Empirical	Empirical
Bath temperature	7° C	7° C	7° C
Water level RUN)S220 E210/E220	Level 8 (S-Series)- Level 5 (E-Series)	Level 5 (S-Series) NA	Level 12 (S-Series)- Level 6 (E-Series)

CONTACT

Please contact Covaris at Application Support (ApplicationSupport@covarisinc.com) if you have any questions.