PROTOCOL

truXTRAC™ Protein Extraction Buffer TP

Adaptive Focused Acoustics™ (AFA) - enhanced reagent for maximal recovery of total proteins

Product PN 520103

Contents

INTENDED USE

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

INTRODUCTION

Covaris **Protein Extraction Buffer TP** (PN 520103) contains urea, thiourea, and a zwitterionic detergent for total protein extraction and maximizing total protein yields from cells and tissues. Urea hydrolyzes rapidly in aqueous solution producing ammonium and cyanate ions which can risk protein carbamylation. Carbamylation modifies amino acids and potentially blocks sites of trypsin digestion and otherwise convoluting MS analysis. Protein Extraction Buffer TP is provided as a stable dry chemical blend to be reconstituted fresh at the point of usage with the below instructions. An ion-exchange resin is included to yield the highest purity reagent. Conductivity of the freshly prepared reagent is typically less than 10 µS/cm making it ideal for applications such as isoelectric focusing (IEF) and two-dimensional gel electrophoresis (2DGE). Also included are a protease inhibitor cocktail and EDTA to preserve protein integrity.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010392	A	4/17	As released

KIT CONTENTS

Bottle IEX	Dry chemicals mixture, includes ion-exchange resin
Bottle TP	Diluent to make 25 mL of Buffer TP
Vial H	0.25 mL of 100X Protease Inhibitor cocktail in DMSO
Vial E	0.25 mL of 100X EDTA (optional)

SDS INFORMATION IS AVAILABLE AT http://covarisinc.com/resources/safety-data-sheets/

STORAGE

The kit is shipped at ambient temperature but should be stored at 4-8°C upon receipt. Before use, the Protein Extraction Buffer TP is reconstituted with the provided diluent. Freshly prepared reagent is stable at 4°C for one week. Unused portions can be aliquoted and stored at -80°C for 6 months.

PREPARATION OF KIT REAGENTS

1. Transfer the entire contents of Bottle TP to the solids in Bottle IEX and mix gently until completely dissolved.

2. Incubate at room temperature with gentle mixing for at least 30 minutes to allow complete reconstitution and equilibration.

3. Remove the ion-exchange beads by filtering the reagent through a 0.45 micron filter back into Bottle TP.

4. The Protease Inhibitor Cocktail (100X) should be added fresh each time at the point of sample preparation to a final 1X concentration

5. Add 250 µL of 100X EDTA from Vial E for a final 1X concentration.

6. The reduction of protein disulfides is performed after AFA. Buffer TP is compatible with DTT, TBP, TCEP, and DE Streak Reagent.

NOTE: DO NOT ADD REDUCING AGENTS PRIOR TO AFA

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

NOTE: Temperatures below 16° C may cause precipitation of urea and detergent

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)	75-150 Watts	100-150 Watts	50-125 Watts
Duty Factor	10%	5%	10%
Cycles per burst	200	200	200
Processing Time	Empirical	Empirical	Empirical
Water level (RUN)* S2/S220 E210/E220	Level 8 (S-Series)- Level 5 (E-Series)	Level 5 (S-Series) - NA	Level 12 (S-Series)- Level 6 (E-Series)

REFERENCES

1. Smejkal, G.B., et al., Anal. Biochem. 363 (2007) 309-311.

CONTACT

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