

truXTRAC™ Protein Extraction Buffer SuperB

Adaptive Focused Acoustics™ (AFA) - enhanced reagent for
Native protein extraction

Product PN 520112

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INTENDED USE

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

INTRODUCTION

Covaris **Protein Extraction Buffer DF** (PN 520112) provides optimized reagents that significantly enhance protein yields while preserving their native conformation, antigenicity, and biological activity. The reagent contains a proprietary non-detergent sulfobetaine (NDSB) that supercharges the reagent, doubling protein yields from tissues. The reagent does not use polyethylene oxide series detergents, such as Triton X-100, which have high micellar molecular weights and are difficult to remove by dialysis. Unlike detergents, NDSBs do not form micelles and are easily removed. Moreover, NDSBs have been shown to prevent protein aggregation (1), facilitate protein refolding (2) and enhance protein antigenicity (3). Proteins extracted in Protein Extraction Buffer SuperB are directly compatible with immunoassay, protein assay, and Blue Native PAGE. Since the reagent does not diminish protein binding, it is directly compatible with most affinity enrichment schemes.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010390	A	4/17	As released

KIT CONTENTS

Bottle SB (SuperB)	Ready to use Protein Extraction Buffer SuperB, 25 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 SB is ready to use. Do not dilute prior to use.

2. When applicable, add Protease Inhibitor Cocktail (Vial H) to Buffer SuperB 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 SuperB.

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

Table 1

	milliTUBE	t-PREP	microTUBE
Volume	1 mL	250 μ L	130 μ L
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)* 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. Collins, T., D’Amico, S. Georgette, D., Marx, J.C., Huston, A.L., Georges Feller, G. Anal Biochem 352 (2006) 299–301.
2. Vuillard L., Rabilloud T., Goldberg M.E. Eur. J. Biochem. 256 (1998) 128-35.
3. Goldberg, M.F., N. Expert-Bezancon, L. Vuillard, T. Rabilloud., Fold. Des. 1 (1996) 21-27.

CONTACT

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