

truXTRAC™ Protein Extraction Buffer DF

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

Product PN 520093

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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 520093) is an efficient detergent-free protein extraction buffer compatible with downstream analyses such as LC/MS, IEF, and 2D gel electrophoresis. The urea-based Protein Extraction Buffer DF includes EDTA, protease inhibitors, and NaCl and is a viable alternative to detergent containing reagents. The AFA compatible reagent enhances protein extraction using Covaris' Focused Ultrasonicators. With the diversity of downstream proteomics applications, extraction buffer selection is typically based on the specifications of the intended downstream analysis technique.

REVISION HISTORY

Part Number	Revision	Date	Description of change
010391	A	4/17	As released

KIT CONTENTS

Bottle DF	Blended dry chemicals
Bottle D	Diluent to make 25 mL of Buffer DF
Vial H	0.25 mL of 100X Protease Inhibitor cocktail in DMSO
Vial N	0.75 mL of concentrated NaCl solution (optional)

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

STORAGE

Dry components can be stored at 4°C for one year. Reconstituted reagent should be used within one week or aliquoted and frozen at -80°C for 6 months.

PREPARATION OF KIT REAGENTS

Urea rapidly hydrolyzes in aqueous solutions, negatively affecting performance of urea based reagents over time, the optimized Protein Extraction Buffer DF is provided as a stable dry chemical blend for reconstitution prior to use.

1. Reconstitute the contents of Bottle DF by adding the entire contents of the provided diluent in Bottle D, mix gently until completely dissolved.

NOTE: DO NOT RECONSTITUTE WITH WATER

2. Add the entire contents of Vial N to the reconstituted Bottle DF. Final NaCl concentration will be 75 mM. Omit NaCl if the intended downstream application is sensitive to salts (e.g. IEF).

3. Add Protease Inhibitor Cocktail 100X (Vial H) to the prepared Buffer DF immediately before use to a final 1X concentration. Add the Protease Inhibitor Cocktail only to the buffer being used immediately.

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 DF 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 DF is compatible with Bradford and similar protein assays to determine the protein concentration. Buffer DF 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 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)	75-150 Watts	100-150 Watts	50-125 Watts
Peak Incident Power (M220)	50 -75 Watts	50 -75 Watts	50 -75 Watts
Duty Factor	10%	5%	10%
Cycles per burst	200	200	200
Processing Time	Empirical	Empirical	Empirical
Bath temperature	18-22° C	18-22° C	18-22° C
Water level (RUN)* S2/S220 E210/E220	Level 8 (S-Series) Level 5 (E-Series)	Level 5 (S-Series) NA (E-Series)	Level 12 (S-Series) Level 6 (E-Series)

CONTACT

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