truPREP® Protein 12x8 Strip Kit
Mammalian Cells (Lysis, Extraction, Digestion)

PN 520354

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Introduction

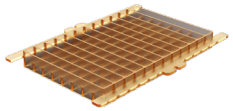
The ***Covaris truPREP Protein 12x8 Strip Kit – Mammalian Cells (Lysis, Extraction, Digestion)*** enables fast and reliable protein sample preparation from cell pellets, resulting in peptides for downstream LC-MS analysis. The comprehensive sample preparation process ensures consistency and reproducibility in a streamlined, single-consumable workflow. The kit accommodates a range of initial cell counts, from 1,000 up to 500,000 cells, offering flexibility and scalability in your experimental design. Developed with the Protein Aggregation Capture (PAC) workflow in conjunction with the Covaris R230 Focused-ultrasonicator, this kit leverages Covaris' Adaptive Focused Acoustics (AFA) technology to optimize efficiency and reduce turnaround time.

Kit Contents

This Kit contains reagents and consumables for up to 96 samples and includes method files and plate definitions for the R230 instrumentation. For accuracy and ease of use, it is recommended that samples are prepared in batch sizes of 16 (two strips) at a time. This kit has been developed and verified with the use of Promega® Trypsin Gold for protein digestion. The trypsin enzyme is not included in the kit and must be supplied by the end user.

Instrumentation and Accessories

The following Covaris instruments and accessory items are necessary to utilize and run this kit. Plate definitions and method files can be downloaded on the Covaris website: https://www.covaris.com/truprep-protein-12x8-strip-kit-mammalian-cells-lysis-extraction-digestion.

Product Name	Part #	Instrument or Accessory	Image
R230 Focused-ultrasonicator	500620	Instrument (not included in kit)	
PSU Rack R230 TPX Plate & 130 Plate (Referred to as R230 Rack in protocol)	500750	Accessory (not included in kit)	
PSU Rack R230 8 AFA-TUBE TPX Strip (Referred to as R230 Accessory Rack in protocol)	500723	Accessory (not included in kit)	
8 microTUBE Strip Prep Station (Referred to as Prep Station in protocol)	500327	Included in Kit	
8 AFA-TUBE TPX Strip (12) (Referred to as Strips in protocol)	520292	Included in Kit	
8 AFA-TUBE TPX Strip Caps (Referred to as Caps in protocol)	500639	Included in Kit	

Items Provided in the Kit

Kit Component	Part #	Volume	Storage Temperature
Magnetic Bead Mixture	190880	2.2 mL	2-8 °C
Lysis Buffer	190882	3 mL	RT
Reducing Agent	190883	1 vial	-20 °C*
Alkylation Reagent	190884	12 ampules	RT
Wash Buffer Diluent	190881	2 x 3 mL	RT
Elution Buffer	190885	3 mL	RT
8 microTUBE Strip Prep Station (Prep Station)	500327	2	N/A
8 AFA-TUBE TPX Strip (Strips)	520292	12	N/A
8 AFA-TUBE TPX Strip Caps (Caps)	500639	12 each	N/A

*Item can be stored at room temperature (RT) prior to reconstitution.

Items Supplied by End User

- 100% Ethanol
- 100% Acetonitrile
- 1x PBS
- Trypsin – Recommended Promega Gold (PN V5280)
- Manual or repeating pipettor
- Heat block with lid
- Magnetic Separator – Recommended Permagen® 0.2 PCR Strip Magnetic Separator (PN MSR812)
- Transfer plate – low binding plate with cover
- 1 mL storage tubes

Workflow

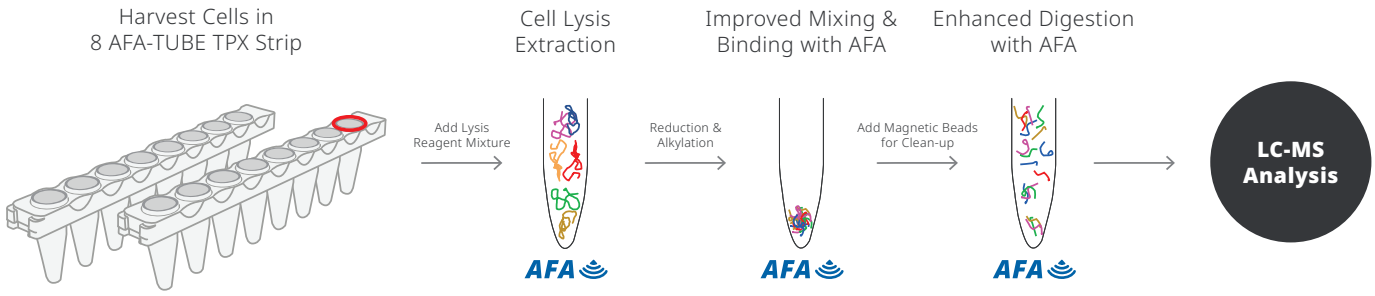


Figure 1. Cells harvested in the 8 AFA-TUBE TPX Strip. Steps shown in single well of Strip.

Instrument Setup

Refer to the R230 Instrument Manual (https://www.covaris.com/wp/wp-content/uploads/pn_010528.pdf) and R230 User Guide (https://www.covaris.com/wp/wp-content/uploads/pn_010480.pdf) for complete setup.

1. Turn on R230 Focused-ultrasonicator prior to starting reagent preparation step.
2. Check for SonoLab software updates and use the latest available version. Plates may require a SonoLab update to run protocol. Current versions of SonoLab can be found at https://www.covaris.com/r-series/.
3. Install R230 Rack (PN 500750) into the R230 if not already installed. This rack will remain installed in the R230 throughout the entire workflow.
4. Go to https://www.covaris.com/truprep-protein-12x8-strip-kit-mammalian-cells-lysis-extraction-digestion to select the appropriate plate method and installation instructions for each of the following procedures and install them on the R230 Focused-ultrasonicator prior to processing samples:
    - truPREP PN 520354 Cell Lysis (***"Cell lysis" in document***)
    - truPREP PN 520354 Mixing and Binding (***"Mixing and Binding" in document***)
    - truPREP PN 520354 Digestion (***"Digestion" in document***)
5. Follow steps on SonoLab for installation of the downloaded methods and plate definitions.
6. Once installed, select the "Cell Lysis" method from the drop-down menu to allow for the water bath temperature to equilibrate and reach set point.

Reagent Preparation

Reducing Agent (PN 190883)

Prior to beginning running samples and protocol, identify the Reducing Agent (PN 190883). Reconstitute the Reducing Agent by adding 5 mL of diH_20. Pipette and/or vortex thoroughly to reconstitute powder. Once reconstituted, aliquot 400 µL volume into 12 x 1 mL tubes. Keep working stock on ice throughout the workflow. Freeze at -20 ˚C any vials that will not be utilized at the time of the experiment. One aliquot of 400 µL will be sufficient for running 1 Strip of 8 samples.

Magnetic Beads (PN 190880)

Prior to running samples and protocols, identify the Magnetic Bead Mixture.

- Place provided magnetic bead mixture on recommended magnet to separate bead mixture from storage buffer.
- Remove storage buffer and then add 2.5 mL of diH_20 to magnetic bead mixture and pipette thoroughly. For volume accommodation, it is recommended for this process be done in a 5 mL tube.
- Place mixture on magnetic rack to isolate the beads from the diH_20. Remove supernatant (repeat 2 times).
- Reconstitute mixture in 2.5 mL of diH_20. Store at 4 ˚C for up to 3 weeks.

Wash Buffer Diluent Preparation

- Prepare wash buffer directly before use (see Digestion).
- Add 20 mL of 100% EtOH to the Wash Buffer Diluent (5 mL). Two bottles are provided with the kit. One bottle contains enough reagent for 6 strips. Formulate one bottle at a time unless all strips are going to be processed in a 7-day time frame.

Promega Trypsin Gold Reconstitution (Provided by customer)

- This kit was developed and optimized with Promega Gold (PN V5280), which is thus recommended for running this kit. Reconstitution directions below are for PN V5280.
- Pipette 100 µL of 50 mm acetic acid directly onto 100 µg of lyophilized Trypsin Gold.
- Pipette mix gently around the bottom of the vial to reconstitute all traces of powder.
- Incubate for 10 minutes at room temperature.
- Aliquot into fresh tubes and freeze at -80 ˚C.
- Thaw Trypsin Gold at room temperature, then store on ice until use.
- Trypsin Gold may be refrozen but should not exceed 5 freeze/thaw cycles.

Lysis with Adaptive Focused Acoustics (AFA)

Grow selected adherent or suspension mammalian cells to desired confluence/cell count. This kit supports a cell range of 1,000–500,000 cells per well. Wash the desired number of cells before pelleting, then discard wash buffer. For one 8-Strip, resuspend the cell pellet in 220 µL 1X PBS and aliquot 25 µL into each well. Pipette 25 µL Lysis Buffer into each well and attach strip caps.

1. Ensure that R230 Rack (PN 500750) has been installed prior initiating the “Lysis” method.
2. Turn on R230 Focused-ultrasonicator and load “Cell Lysis” to prepare for the Lysing step.
3. Verify that “Cell Lysis” method has been selected from the drop-down menu.
4. Place the Strip in the Prep Station.
5. Aliquot 25 µL of washed prepared cell pellets into each well of the Strip.
6. Add 25 µL of Lysis Buffer to each well.
7. Affix Caps to the Strip.
8. Remove the Strip from the Prep Station and place into Accessory Rack (PN 500723). This Rack rests on top of the installed R230 Rack (PN 500750).
9. Insert the Strip with samples into the R230 Focused-ultrasonicator.
10. Run the “Cell Lysis” program on the R230 Focused-ultrasonicator.
11. Once AFA treatment is completed, remove Strip and tap or briefly centrifuge the Strip to ensure all droplets have settled in the bottom of the well.
12. On the R230 select “Mixing and Binding” protocol for Instrument to prepare for binding step.
13. ***Optional Step***: At the completion of the lysis portion of this protocol, a sample volume of 5 µL in duplicate (total of 10 µL per sample) can be removed from your lysate to quantify protein yield with a BCA assay. It is recommended that the removed sample be diluted 1:5 with diH_20 prior to running the BCA assay. We recommend utilizing the Pierce™ BCA Protein Assay Kit (PN 23227 - Thermo Fisher Scientific) and running the assay according to the provided protocol and directions. Peptide yields for these sampled wells may have lower yield than remaining non BCA tested wells.

Reduction and Alkylation

1. Pre-heat thermocycler or heat block to 50 ˚C.
2. Ensure the Reducing Agent (PN 190883) that has been reconstituted is stored on ice.
3. Remove the Strip from the R230 Accessory Rack.
4. Place Strip into Prep Station.
5. Remove the Caps from the Strip.
6. Add 2 µL Reducing Agent to each well.
7. Affix the Strip Caps.
8. Remove capped Strips from Prep Station and place Strips on heat block or in thermocycler for 1 hour at 50 ˚C.
9. After the hour incubation, remove from heat block and tap or briefly centrifuge the Strip to ensure all droplets have settled in the bottom of the well.
10. Allow to cool to room temperature for 10 minutes.
11. Place Strip into Prep Station and remove Caps.
12. Reconstitute the Alkylation Reagent (PN 190884) by adding 100 µL of diH_20 to the 9.3 mg ampule (light sensitive). Each ampule contains enough reagent for 8 samples or one Strip.
13. Add 5.8 µL Alkylation Reagent to each well and pipette to mix.
14. Discard any excess Alkylation Reagent.
15. Replace the Caps and store at room temperature in the dark for 20 minutes.
16. Quench reaction by adding 5 µL of Reducing Agent (PN 190883) to each well.

CAUTION: Do not attempt to download SonoLab 10.0 if your Instrument does not have compatible firmware versions installed.

Purification

1. Load method on the R230 for “Mixing and Binding”.

> **NOTE:** Covaris Lysis Buffer is detergent-based, hence the need to purify samples to ensure LC-MS compatibility.

2. If using frozen samples, thaw to room temperature before proceeding with purification and digestion steps below.
3. Ensure the magnetic bead mixture has been washed as outlined in the Reagent Preparation step listed above.
4. Place the Strip into the prep station and remove the Caps.
5. Add the appropriate volume of magnetic bead mixture to each well (based on chart below). Pipette mix 10x.

Cell Count	1,000–50,000	50,000–100,000	100,000–500,000
Bead Mixture	5 µL	10 µL	20 µL

6. Add the appropriate volume of 100% EtOH to each well (based on chart below).

Cell Count	1,000–50,000	50,000–100,000	100,000–500,000
100% EtOH	65 µL	70 µL	80 µL

7. Replace the Strip Cap.
8. Insert the Strip into the R230 Accessory Rack and place it into the R230.
9. Start method for ”Mixing and Binding”.
10. Remove the R230 Accessory Rack and Strips from the R230 at completion of “Mixing and Binding” run.
11. Select the “Digestion” protocol on the R230 and load the protocol. This will allow the water bath to equilibrate for the trypsin digestion process as described below.
12. Remove the Caps from the Strip.
13. Place the Strip on the magnetic separator for 2 minutes. After 2 minutes, remove the supernatant and discard.

> **NOTE:** Care should be taken so as not to disturb the bead pellet while pipetting.

14. Add 200 µL to each well of the prepared wash buffer (as outlined in the Reagent Preparation step) and remove the Strip from the magnet before pipetting the mixture to resuspend the beads.
15. Place the Strip back on the magnetic separator and incubate for 2 minutes.
16. Repeat steps 13–15 one additional time.
17. Add 180 µL 100% Acetonitrile (not included) and remove the Strip from the magnet and pipette beads to resuspend.
18. Incubate on the magnet for 2 minutes.
19. Remove supernatant while the Strip is on the magnet (make sure that all Acetonitrile is removed without disturbing bead pellet).

Digestion with AFA

1. Before beginning this section, ensure that the "Digestion" protocol is loaded on the R230.
2. Reconstitute your Promega Trypsin Gold (if using an alternative Trypsin, a final concentration of 1mg/mL is necessary for the dilution tables below).
3. Prepare Trypsin/Elution Buffer mixture (based on cell count chart below).
4. Resuspend beads in 22 µL of Trypsin/Elution Buffer per well.
5. Place the Caps onto the Strip.
6. Place the Strip into the R230 Accessory Rack.
7. Place the R230 Accessory Rack with Strip into R230 and begin the "Digestion" protocol.
8. After AFA treatment, remove R230 Accessory Rack from the the R230. Gently tap Strips on bench top to collect any droplets that may have splashed on Cap during AFA treatment.
9. Incubate the Strip on the magnet for 2 minutes.
10. Remove the Caps.
11. Transfer supernatant into fresh tubes or low binding plate.
12. Seal plate or cap tubes.
13. Store at -80 ˚C for 3 weeks and/or proceed to process with LC-MS analysis for the eluted peptides.

Cell Count	1,000–50,000	50,000–100,000	100,000–500,000
Trypsin	8.8 µL (reconstituted)	17.6 µL (reconstituted)	88 µL (reconstituted)
Elution Buffer	184.8 µL	176 µL	105.6 µL

Calculations are based on 8 samples/1 Strip.

14. ***Optional Step***: At the completion of the digestion portion of this protocol, a sample volume of 5 µL in duplicate (total of 10 µL per sample) can be removed from your digest to quantify peptide yield with a peptide assay. It is recommended that the removed sample be diluted 1:1 with diH_20 prior to running the peptide assay. We recommend utilizing the Pierce™ Quantitative Fluorometric Peptide Assay (PN 23290 - Thermo Fisher Scientific) and running the assay according to the provided protocol and directions.

Support and Technical Assistance

Tech Support: Ongoing assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- **Telephone:**
  - US & APAC: +1 781.932.3959, during the hours of 8:30 a.m. to 5:00 p.m. (EST), Monday through Friday
  - EU: +44 (0)845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m. (GMT), Monday through Friday
- **E-mail:**
  - US Customer Service: customerservice@covaris.com
  - EU/UK Customer Service: emeacustomerservice@covaris.com
  - APAC Customer Service: APACcustomerservice@covaris.com
  - Service and Instrumentation: techsupport@covaris.com
  - Applications: applicationsupport@covaris.com

US: +1 781.932.3959
customerservice@covaris.com

EU: +44 (0)845 872 0100
emeacustomerservice@covaris.com

APAC: +86 137 6427 6714
APACcustomerservice@covaris.com

Applications
applicationsupport@covaris.com

Service and Support
techsupport@covaris.com

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