UT SOUTHWESTERN PROTEOMICS CORE

IN-SOLUTION DIGESTED SAMPLES SERVICE

IMPORTANT – WARNINGS & RESTRICTIONS FOR IN-SOLUTION SUBMISSIONS

Various compounds, particularly the most commonly used detergents in laboratory work, are incompatible with mass-spectrometry analysis. When samples are run in-gel the gel acts as an effective filter to remove these molecules. When samples are prepared in-solution, these contaminants *MUST* be avoided. Failure to adhere to the advice in this document can result in serious down-time for our instrumentation, due to the need to clean or replace contaminated parts. This is very time-consuming for core staff, expensive, and not compatible with the operation of a core-facility.

All in-solution digested samples will be checked for contamination by running them on our LTQ platform, which is dedicated to QC. If clean they will be analyzed on our main high-resolution service instrument. If found to be contaminated you will be informed, and the following measures taken:

For the first contaminated sample: you will be required to meet with core staff, provide complete descriptions of your experiment and sample prep, and agree to follow all advice given.

If further samples are contaminated: you will no longer be permitted to submit in-solution samples.

Use a gel-based workflow whenever possible!

When submitting in-solution digested samples we *REQUIRE* that you:

- Contact the core via ProteomicsCore@UTSouthwestern.edu in advance of beginning sample prep, giving an overview of your experiment, and listing reagents used.
- Do not use ***ANY*** detergents in ***ANY*** step of sample-prep other than RapiGest SF (Waters). This includes, but is not limited to a ban on: SDS, CHAPS, NP-40, Triton-X100 (or related). The p*rohibition of other detergents includes lysis, solubilization, IP steps in your experiment.*
- Perform all sample preparation in your own laboratory. Unlike in-gel samples we do not perform the digest for you.
- Follow the digestion protocol given in this document, and pay attention to general guidance on page 2.
- Purchase all of the required reagents, and dedicate them to your in-solution proteomics work. Contamination commonly occurs from sharing of reagents with others not aware of the contamination risks. The core does not share any reagents, due to the risk of contamination
- Purchase new glass and plastic-ware to be dedicated to your in-solution proteomics work. This equipment must never be washed with detergents.

We recommend that you:

- Arrange a meeting with core staff prior to your experiment.
- Think carefully whether a gel-based approach can be used. You may need more tissue / cells / antibody etc. but it will almost certainly be cheaper, quicker, and with less risk of failed submissions due to contamination.

CONTACT US IF YOU ARE UNSURE ABOUT ANY STEP IN YOUR EXPERIMENT

GENERAL INSTRUCTIONS / GUIDANCE

Waters RapiGest SF

The only detergent that may be used for in-solution work is RapiGest SF, produced by Waters. This is an acid-cleavable detergent. When samples are acidified after tryptic digestion it breaks down into by-products that can be removed / are compatible with downstream MS analysis. Other manufacturers produce similar products, but we have experience of, and protocols for RapiGest SF.

Tubes and Containers

When working with proteins in-solution substantial amounts of material can be lost via binding to surfaces. We recommend Eppendorf Protein LoBind micro centrifuge tubes. High quality micro centrifuge tubes are required. Low grade tubes have, in some cases, resulted in contamination of samples via leached polymers and plasticizers.

All bottles and other containers used should be purchased for, and dedicated to, in-solution proteomics work. Do not wash with detergent. Do not share with others in your lab for non-proteomics purposes.

Pre-Digest Lysis / Solubilization Steps

SDS, NP-40, TWEEN, CHAPS, Triton-X100, or any other detergent ***MUST NOT*** be used in any step, including lysis or protein solubilization steps. Substitute RapiGest SF for these detergents, or use detergent-free mechanical lysis. Note that by the point of digestion you should have ≤0.5% RapiGest SF to avoid inhibition of tryptic activity - unless working with extremely hydrophobic proteins, which may benefit from increased surfactant despite lower digestion activity. At 0.5% concentration tryptic activity is 87%.

Co-IP

SDS, NP-40, TWEEN, CHAPS, Triton-X100, or any other detergent ***MUST NOT*** be used in any step, including IP. Substitute RapiGest SF for these detergents. Many publications use RapiGest in IP. Consult the literature for concentrations that have been effective in similar experiments.

Tryptic Digestion

Follow the digestion protocol provided on the next page. Pay particular attention to the need to make up fresh IAA solution for each digest, and perform alkylation in the dark. Do not exceed the incubation times or temperatures for the TCEP & IAA steps as undesirable protein modifications can occur.

You must use sequencing grade enzyme, and high purity reagents. As with your containers, these reagents should be purchased for, and dedicated to, in-solution proteomics work. Do not share with others in your lab for non-proteomics purposes.

Sample Submission

Your final submission to the core should be a peptide mixture in a micro centrifuge tube, labelled and entered in the online system as normal. All in-solution samples are run on our QC platform to check for contamination, prior to the main analysis. This may lead to a longer turnaround vs in-gel submission, depending on the sample queue.

PROTOCOL – UTSW Proteomics Core In-Solution Digestion with RapiGest SF

Reagents

Use high-purity reagents and sequencing grade enzyme.

1. Trifluoroethanol (TFE)
2. Urea
3. Thiourea
4. Tris(2-carboxyethyl)phosphine (TCEP)
5. Iodoacetamide (IAA)
6. RapiGest SF (Waters)
7. Trypsin (sequencing grade, Promega) ~0.5mg/ml
8. Trifluoroacetic acid (TFA)

Stock Solutions

1. Digestion Buffer (1x)
    a. 100 mM Tris pH 8.0
    b. 50% TFE
    c. 6M urea
    d. 2M thiourea
    e. 0.1% Rapigest SF
2. TCEP Stock Solution (25x)
    a. 200 mM TCEP solution in water
3. IAA stock solution, made FRESH each time (25x)
    a. 300 mM IAA solution in water
4. Dilution Buffer
    a. 100 mM Tris pH 8.0
    b. 10 mM CaCl2

Procedure

1. Add digestion buffer to the protein pellet to a final concentration of 1µg/µl, vortex and keep at 37°C for 30 min.

2. Add TCEP stock solution to a final concentration of 1x, vortex and keep at 37°C for 30 min.

3. Add IAA stock solution to a final concentration of 1x, vortex and keep at room temperature for 30 min *in the dark*.

4. Dilute the digestion 10x using the dilution buffer.

5. Add trypsin in a 1:50 ratio of trypsin:protein and incubate overnight at 37°C with shaking or end-over-end rotation.

6. Add another 1:50 ratio of trypsin:protein and incubate at 37°C for 2 hours.

7. Stop the digestion and cleave RapiGest SF by adjusting the pH to <2 using TFA (approx. 0.5%) and incubating for 30-45 minutes at 37°C. Slight cloudiness should be observed

8. Centrifuge at 13,000rpm for 10 minutes. Some precipitation of RapiGest cleaved products may be observed.

9. Transfer the solution to another micro centrifuge tube.

10. Enter samples details online, bring sample to the Proteomics Core Facility with an IDR.

Notes / Modifications

When working with very hydrophobic proteins RapiGest SF concentration may need to be increased above 0.1%. At higher concentrations tryptic activity is reduced. Trypsin has 87% activity with a 0.5% RapiGest.

RapiGest literature indicates the surfactant can be used to speed-up tryptic digestion. However, overnight digestion is safest to achieve complete digestion, particularly with hydrophobic membrane proteins and higher concentrations of surfactant.

Cautions

Use high purity reagents and sequencing grade enzyme. Do not share with others for non-proteomics work.

Use dedicated glass containers for reagents. Do not wash with detergent.

Consider using Eppendorf Protein LoBind tubes to minimize loss of protein due to binding to plastic. This may be particularly important for low abundance work.