S-Trap™ FAQ

S-Trap General

Can the S-Trap protocol be run without SDS?
S-Trap sample processing is predicated on SDS. Do not attempt S-Trap sample processing without SDS. 2% SDS is the minimum.

Is there an expiration date for the S-Trap columns?
2 years.

Is the S-Trap compatible with all proteins?
Yes, with the single exception of alcohol soluble proteins, which we have observed only in plant seeds samples. (You can verify that your sample has no methanol soluble proteins by speed-vacing the flow-through and washes of the S-Trap and running that fraction on an SDS-PAGE gel; we have not observed this with anything outside of seeds.) If you are studying plant seeds, remember that prolamin storage proteins are alcohol soluble. These include the gliadins of wheat, the avenins of oats, the zeins of corn, the hordeins of barley and secalins of rye. In addition, while glutelin proteins are typically oligomerized via interchain disulfide bonds and thus insoluble, they become alcohol soluble when reduced.

Do S-Traps trap low molecular weight proteins?
Yes, especially if they are in a complex mixture with many other proteins which tend to carry them into the protein trap. However, proteins have extraordinarily diverse properties and if you're working with a purified protein, there is a chance that it has unusual properties like methanol solubility (see below).

Do S-Traps trap peptides?
No.

What is the reproducibility of S-Trap sample processing?
Typically < 10% CVs for replicate digestions.

What buffer components will the S-Trap remove?
Almost everything! Urea (even 8M), salts, glycerol, PEG, other detergents, Ficoll, tween, triton, Lamelli loading buffer … Just make sure to add SDS to 2% – 5% final concentration (and follow the S-Trap protocol. The one thing to avoid is 6 M guanidinium chloride: it is positively charged, SDS is negative and when you put the two together, they form an insoluble precipitate. If you've protocols with 6 M GuHCl, swap it out for 5% SDS. As with everything, we always recommend testing your specific conditions in particular for important samples.

I have a detergent-containing buffer. The detergent isn't SDS. Can I still use S-Traps? What should I do?
Add SDS to your buffer to at least a 2% w/v final concentration. Then proceed as normal: add phosphoric acid, then binding buffer and everything will be great! Like with other buffer components, if you run into a buffer component which is "sticky" and appears to not be fully removed by three washes, simply wash more.

Is S-Trap compatible with detergents such as Rapidest and ProteaseMax?
Yes. However, do not use ProteaseMax at 47 C as its auto-degradation is too quick.

I have a cell lysate with 6 M guanidium hydrochloride and 25 mM Tris [pH 8]) containing 5 mM tris(2-carboxyethyl)phosphine (TCEP) and 5.5 mM chloroacetamide (CAA). Would it be fine to add a final volume of 2% SDS in order to run the samples into S-trap?
SDS and GuHCl make an insoluble ionic salt which precipitates. Thus, substitute SDS for protocols requiring GuHCl. If you have samples which already contain GuHCl, additional washes may remove the precipitant, however it is best to simply avoid GuHCl.

Can S-Trap remove contaminants such as Trizol, Phenol, Iodixanol, and N-Octyl-Beta-Glucopyranoside, NP-40 (Nonident P-40) and Sodium DeoxyCholate (SDC)?
Yes. S-Trap can remove all of these.

Can S-Trap remove paraformaldehyde and HE staining solution?
Yes. Use the HYPERsol protocol available on the website.

Can I use S-Trap to remove SDS from my peptide solution?
No. Peptides don't bind to S-Trap, only proteins. Peptides will typically appear in the SDS-containing flow through. Use cation exchange.

Protein Concentration

How do I match my protein concentrations when I solubilize everything in 5% SDS?
Use a BCA assay and not Bradford: BCA is not sensitive to detergents and Bradford is. Note that the original BCA assay is not compatible with reducing agents. There exist, however, reducing agent compatible BCA assays, e.g. BCA-RAC assay from Pierce. You can also use amine-reactive fluorogenic reagents such as CBQCA (3-(4-carboxybenzoyl)-2-quinolinecarboxaldehyde) and also absorbance at 280 nm. We use the standard Pierce BCA assay and get outstanding results: apply 20 uL of sample in various dilutions (i.e. dilute e.g. 1:1, 1:3, 1:10, 1:30 into 5% SDS lysis buffer (without reducing reagents) along with a standard curve in the same buffer and plate in triplicate) into a 96 well plate avoiding the outer row and column. To all samples, then add 200 uL of working reagent with a multichannel pipette (go fast and accurately). Then place the plate on a 95 C block and wait until you get a nice deep purple color at the e.g. 2 mg/mL max protein concentration. Measure and plate this. You will find the curve to be extremely linear.

Extraction/Lysis/Solubilization

What is the best way to lyse my cells?
If you want to simultaneously lyse your cells and instantly stop all enzymatic activity, "freezing" the state of biology, heat 1X lysis/solubilization buffer (5% SDS, 50 mM TEAB pH 7.55) to 95 C and add TCEP to 5 mM. Squirt this on your cells and sonicate (better: process with AFA) to sheer the DNA. Let cool to room temperature, add MMTS to 15 mM, let stand for 30 min (or 10 min at 37 C) and you're ready to process. Note that this approach is also one of the best ways to prevent loss of PTMs by endogenous enzymatic activity.

My sample is not going into solution. What should I do?
For particularly difficult samples, be sure to start with samples that are as finely pulverized as possible. Especially for tough samples like tissues, either find and use a bead beater under liquid nitrogen or use a Covaris cryoPREP to pulverize it. Alternatively, for larger scales and absolute maximum pulverization, dice your sample (preferably frozen), lyophilize it and use a laboratory-scale jet mill. Note the losses here will be higher.
If you are sample limited and/or want to improve your recovery, use a Covaris AFA unit which is well suited to small sample amounts, or a cryogenic bead beater with frozen 5% SDS. For AFA, place the sample in 5% SDS and immediately AFA treat until the sample is fully disaggregated. Samples are kept isothermal typically at 4 C. For cryogenic bead beating, freeze 5% along with the sample, make sure the bead(s) are able to move, and pulverize until a free-flowing powder results. In both cases, 5% SDS will coat the surfaces of tubes, tips and beads, limiting loss. Surfaces can be washed if desired with additional buffer.
If a Covaris AFA unit or cryogenic bead beater is not available, probe sonication is an option. Be careful, however, to not explode your sample out the top of the tube. Alternatively, or additionally, incubate samples with agitation at elevated temperatures (e.g. 55 C for 2 hrs on a heating block or overnight at 37 C on an end-over-end rotator). Note that elevated temperatures can cause sample degradation, even with enzymes like proteases and phosphatases inhibited by 5% SDS.
For tissues and other samples which may be crosslinked through disulfide bonds, we recommend including 5 – 10 mM TCEP during the solubilization step. (Note however that this must be removed before a standard BCA assay or use a reducing reagent compatible BCA assay.)
With extensive solubilization, it may be useful 1) to sparge your solutions with nitrogen or argon and to keep the protein sample under the same atmosphere (to reduce oxidation) and 2) to do an initial extraction (remove the supernatant and keep it) followed by a harsh extraction. Sequential extraction can be useful to 1) preserve the chemical integrity of the proteins which more easily go into solution and 2) to provide different classes of proteins. Frequently, the protein of interest is more concentrated in one of the fractions.

DNA Cleanup

How should I best remove DNA? (Should add PIXUL)

There are two simple and easy ways to remove DNA. First, apply high-intensity sonication with a probe sonicator or most preferably with a PIXUL or Covaris Adaptive Focused Acoustics (AFA) unit. (If you are manually processing samples, make sure to keep the time, intensity and depth of probe contact constant between samples). Alternatively, resuspend your samples in 5% SDS containing 2 mM MgCl2. Then, add a small amount of benzonase by Roche (e.g. 0.5 μL of 250 units per μL into 25 μL). Your DNA will almost instantly be gone. Benzonase maintains activity in 5% SDS with magnesium sufficiently long to remove destroy DNA which then washes through.

PIXUL megasonication and Covaris AFA technologies are a non-contact process which imparts strong acoustic forces in a tightly controlled, isothermal environments. They generate extreme sheer forces which homogenize samples, sheer DNA and force proteins into solution, especially in the presence of 5% SDS. Both PIXUL and AFA are amenable to low input material especially with 5% SDS which coats tubes and tips.

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Do you have any recommendations for sonication settings to shear the DNA?

Use an ultrasonicator, not a probe or bath sonicator. [Add settings.] We recommend the PIXUL sold by Active Motif (a benchtop 96-well plate incubator) and Covaris devices which use AFA (Adaptive Focused Acoustics). Most genomics cores have Covaris instruments that you can use. Probe or bath sonication is NOT equivalent to ultrasonication.

Why should I use an ultrasonicator instead of a probe sonicator?

Probe sonicators are in the kilohertz range whereas ultrasonicators are usually in the gigahertz range. Recall that the energy imparted by a wave is proportional to the square of the amplitude of the wave times the square of its frequency. So ultra sonicators are maybe 0.5 – 3 MHz. Thus, you're looking at a minimum of (500/20)^2 = 25^2 = 625X more energy given by an ultrasonicator at the low end set at the same energy as your puny little probe sonicator. That's pretty significant!

Buffers and pH

Can I use other buffers than TEAB?

Yes. In all buffers, you can use tris in place of TEAB at the same pHes and concentrations. You can also use ammonium bicarbonate in the lysis and digestion buffer. However, you cannot use ammonium bicarbonate in the methanolic S-Trap binding buffer: its solubility is not great enough in 90% methanol. You can use ammonium bicarbonate (or anything else) in the digestion buffer without issue. Remember however: if doing iTRAQ or TMT, you must use TEAB, HEPES or another buffer without primary or secondary amines (cf. Good Buffers).

If I make my own buffers, what can I adjust the pH with?

For the lysis buffer, we recommend using phosphoric acid to adjust the pH as the samples will be further denatured with that acid. For the methanolic S-Trap binding/wash buffer, we recommend phosphoric acid. For the digestion buffer, anything compatible with your protease can be used including whatever acid or base is needed to correctly set the pH. Note that typical digestion buffers of ammonium bicarbonate and TEAB do not require pH adjustment for trypsin. However, buffers made with tris or HEPES free base (or acid) will need pHing.

Can I use another acid than phosphoric acid?

Yes, lactic acid at 3% or citric acid at 4% final concentration.

How essential is the correct pH value?

For the lysis buffer, not important at all. For the binding buffer, important. Note that TEAB is volatile and can change pH if left to sit or stored with a large air headspace. We recommend adjusting the pH of a 1 M solution with 85% phosphoric acid, aliquoting this solution and keeping it frozen.

What is the recommended error range for the pH while preparing S-Trap protocol buffers?

0.05

What is the composition of 2X SDS lysis/ Solubilization buffer?
10% SDS, 100mM TEAB, pH 7.55.

What is the composition of high recovery urea-SDS lysis/solubilization buffer?
5% SDS, 8 M urea, 100 mM glycine* pH 7.55.
TEAB can also be used* in place of glycine.

How do you make S-Trap protein binding buffer called for in the S-Trap protocol?
Adjust the pH of 1 M TEAB to 7.55 with phosphoric acid. Go slowly to change the volume minimally. After adjusting the pH, aliquot e.g. 1.5 mL into 15 mL Falcon tubes. Freeze all the tubes but one. When you need additional binding buffer, add sufficient MeOH to a tube containing 1.5 mL of 1 M TEAB, pH 7.55 to bring the volume to 15 mL.

Is there a quick table for all the buffer compositions, pH, storage and shelf life that S-Trap

Buffer	Composition	pH	Storage	Shelf life
Lysis Buffer	5% SDS, 50 mM TEAB pH 8.5 (bottle is at pH 8.5)	No need to adjust the pH for lysis buffer	Room temperature	At least for a year
DTT Reduction Buffer	20 mM	Do not adjust the pH	Do not store	Make fresh each time
Iodoacetamide Alkylation Buffer	40 mM	Do not adjust the pH	Do not store	Make fresh each time, keep in dark
12% Aqueous Phosphoric Acid	Pre-prepared. Added to the SDS lysate to a final conc. of 1.2% e.g. 2.5 uL to 25 uL	Do not adjust the pH	Room temperature	At least for a year
Binding/Wash buffer	100 mM TEAB (final) at pH 7.55 in 90% methanol. To prepare, adjust the pH of 1 M TEAB to pH 7.55 with 12% phosphoric acid and aliquot 1.5 mL in 15 mL Falcon tubes. Freeze the tubes at -20 C or -80 C except one. For 15 mL of fresh binding buffer, add HPLC grade MeOH to bring the final volume to 15 mL, making a 90% methanol solution.	pH 7.55	4 C	One month
Trypsin digestion Buffer	50 mM TEAB, pH 8.5	Do not adjust the pH	4 C	One month
Elution Buffer 1/ TEAB Wash Buffer	50 mM TEAB pH 8.5	Do not adjust the pH	Room temperature	One month
Elution Buffer 2/ Formic acid	0.2% formic acid in water	Do not adjust the pH	Room temperature	One month
Elution buffer 3/ Acetonitrile	50% acetonitrile in water	Do not adjust the pH	Room temperature	One month

Enzymatic Digestion

Can I use GluC for protein digestion instead of Trypsin?
Yes. Overnight digestion in a humid environment are best. Do not digest at 47 C.

Can I use other enzymes than trypsin in the S-Trap? Are the conditions the same?
Yes. You can use other enzymes. However, the conditions are not the same as for trypsin and you need to standardize the conditions.

Will a 1-hour 47 C digestion give the same results (numbers of identified peptides, missed cleavages, etc.) as an overnight in-solution digestion?
That actually depends on your instrument and how it's run. On a relatively fast 1D run of a complex sample, especially with a machine that is a year or two old, you will probably not see a difference. On a fast and new instruments such as new Orbitrap class machines, by peptide counting, you will see an increase in missed cleavages from ~10% – 15% for an overnight tryptic digest to ~20% – 30% for a 1-hr digest. However ion current tells a different story: if we set to 100% the total amount of ion current in peptides with no missed cleavages generated in an overnight tryptic digest, the corresponding amount of ion current for fully tryptic peptides in a 1-hr digestion is in our experience always >90% with >95% being quite common. This discrepancy follows from the fact that new machines are simply so fast and so good at picking up low level missed cleavages i.e. little peaks. A 1-hr digestion time is thus "95% of the way there" and matches the duty cycle of typical runs (around 1 hr) to the duty cycle of sample preparation. However, you can and should optimize digestion for your samples and workflows. We have people doing everything from 10 min digestions (at 1:1 wt:wt substrate:trypsin to limit deamidation) to overnight digestions (use a water bath to keep columns from drying out, or place a beaker of water in an incubator).

Why 47 C? Does 47 C work for other enzymes?
47 C for 1 hr is for trypsin only and is an intermediate between the S-Trap and digestion buffer warming up, trypsin working very rapidly and then starting to thermally denature to the end of the 1 hr. It was empirically determined for trypsin. Other enzymes require different temperatures and times.

What happens if the columns dry out?
Just rehydrate them with digestion buffer, let them sit for 15 min at 37 C and follow the standard elution procedure. The protein trap is designed to have no affinity for digested peptides; they just need to be solubilized.

How do I remove bubbles from the trap?
The easiest way is to "flick" the tubes. Alternatively you can pipette the digestion solution up and down. Just be sure that all the solution is on the trap and not on the side of the spin column where it will dry out.

Can the columns stay closed during incubation with protease?
The columns cannot be air-tight during incubation with protease and the minis have a vent; the micros need to be left slightly open, however.

Why is it important to cap the S-Trap micro and midi columns loosely?
When you cap the column tightly and leave it at 47 C, it increases the air pressure on top of the column, which forces the digestion solution out of the bottom. Thus, you must allow the pressure to equilibrate.

How to deal with the evaporative loss during digestion at 47 C that is done for a long period of time?
Either perform your digestion in a water bath or put beakers full of hot water in dry incubators to saturate the environment with water vapor. Alternatively, add more digestion buffer e.g. 2X (but keep the amount of trypsin the same).

Peptide Elution and Cleanup

What proportion of peptides come off in each eluate with TEAB, formic acid and acetonitrile respectively?
85% with TEAB, 10% with second elution and remainder with acetonitrile. Hydrophobic peptides will come out predominantly in the acetonitrile elution.

Can I add TMT reagent directly to the eluted peptides without drying them down and resuspending?
TMT labeling is sensitive to pH and water content. Therefore, it is important to dry down the eluted peptides and resuspend to control for both of these factors.

Do I need to clean my peptides before running them?
We generally recommend concentrating the peptides using desalting columns, however, C18 columns and tips have much lower recoveries than most people are aware of: around 50% – 60% in typical use. If you are working with low-level and/or hydrophobic samples, the serial loss over desalting steps, a LC trap column and then an analytical column can result in seeing nothing or almost nothing. We and customers have observed this in particular with exosome samples: the proteins are typically hydrophobic, and membrane bound and often people have only a microgram or two. If you are doing a large-scale digestion for PTM enrichment, you will want to do desalting so that you can dry your peptides down and solubilize them in the binding buffer for your PTM enrichment.

Is C18 desalting still needed for TMT labelling if I use S-Trap kit?
If you are performing iTRAQ or TMT labeling of peptides post digestion, yes. If you are performing protein-level labeling, no. Also see "Do I need to desalt my peptides".

When is it most important to desalt peptides?
Desalting is important before enrichment. e.g. for enrichment by antibodies or phosphoenrichment by IMAC. Desalting is most important for phosphopeptide enrichment: while the peptides come out squeaky clean from S-Traps (i.e. free of everything including detergent and NaCl etc.), they have been exposed to phosphate anions in the denaturation/binding process. The phosphate anions can remain due to ionic interactions with basic groups (RKH), they are replaced with (typically) formate or TFA anions during desalting.

I dried down my peptides and have a larger pellet than I think I should have. What is going on? Or: my LC system is working great, but the peptide chromatography doesn't look right.

Both of these are signs that the protein must be washed more to further remove SDS and/or any buffer components: do further washes. SDS is moderately hydrophobic and will come off C18 columns. Before it does however, it alters the surface to make it more of a cation exchange.

S-Trap Applications

My [favorite] protein is impossible: I can't seem to see it! What to do?

That's a tricky – and addressable – question, especially if your protein exists in another source such as recombinant or through an overexpression system. You must address two basic questions: 1) is your protein getting into your digestion and being observed? and 2) if it makes it in, is it there in sufficient quantity to see in the background of your other proteins and your up-front separation? If you have a protein source, start with a positive control: do an S-Trap digest of, by example, a cell pellet from induced over expression. Verify that you have protein (e.g. you have a large band after overexpression on an SDS PAGE gel, or you have it from a purified source), that you're sure your machine is working (positive control of e.g. HeLa cell tryptic digest) and that your digestion is working (run an SDS-PAGE gel of the input and output of an S-Trap digest). If these all check out but you still can't see your protein by mass spec proteomics, you need to consider possibilities. Biochemical reasons include that the protein is not the expected protein (this happens more frequently than people realize); that it has a limited number of lysines or arginies; or that the peptides are insoluble in buffer A of your LC system, or that they are not coming off your separation column, or of course some combination of these reasons. Bioinformatics search reasons include the protein being absent from the database, the wrong databases being searched, and wrong search parameters (including enzyme specificity, fixed PTMs, etc.). Once you have verified that you can see your protein by proteomics, the next question is one of dynamic range. If you have purified protein, add it back to your sample at 0.1% wt:wt to begin and determine if you can detect it within that background given the current LC separation. Titrate up and down as needed to establish a lower limit of detection (LLD). If this LLD is insufficient to detect your protein at the levels present, you must increase chromatographic separation and/or determine a way to preferentially extract your protein, leaving behind interfering signal. For difficult proteins at particularly low levels, Western blots with extended incubation times may be more practical.

I've got an immunoprecipitation (IP)/I need to do an IP. Now what?

There are a few considerations with IPs including how dirty or clean they are, how much protein has been immunoprecipitated (a function both of how much protein is present and the amount and affinity of antibody, as well as binding conditions) and the signal to noise ratio of capture antibody to target antigen. To improve the cleanliness of IPs, run experiments of increasing wash stringencies monitored by Western and silver stained gel: you should increase stringency so that the background is clear while maintaining clear differences between a control lane (ideally an isotype-controlled antibody without affinity to your target antigen) and the experimental lane. As the S-Trap removes all such buffer component, you can use salts, chaotropes and detergents including polymeric detergents such as tween or triton. This optimization is typically the key to getting reliable IP results and additionally improves the signal to noise for small amounts of captured target protein. To improve the relative amount of captured antigen to capture antibody, you can covalently anchor your antibody (e.g. use BS^3 on protein A/G after Ab binding, or put your antibody on covalently with for example epoxy or CNBr derivatized beads). You can elute the IPed proteins either with 5% SDS (and heating to 95 C as desired; often this helps), which will strip everything especially if reduction and alkylation is done on beads. Alternatively, you can try to use other detergents or conditions which elute the protein(s) of interest and leave behind the "garbage." Cf. Impact of Detergents on Membrane Protein Complex Isolation. http://www.ncbi.nlm.nih.gov/pubmed/29110486. If you didn't elute in SDS, add it to 5% and the SDS will carry the (for example) nanograms of IPed material which you will then efficiently retain with the high recovery protocol. The high recovery protocol uses trypsin itself as both a carrier and a proteolytic enzyme for three reasons: first, without a carrier, the small amount of antigen will be poorly retained; second adding trypsin does not further complicate your spectrum (often tryptic autodigestion products are already in exclude lists); and third, at those very low levels, in an [ES] reaction the substrate is at a very small concentration (and thus rate limiting, rather than the protease), so we must significantly increase the concentration of trypsin for efficient proteolytic digestion.

Someone brought me an immunoprecipitation (IP) in blue Laemmli loading buffer. Can S-Traps help?

Yes. Reduce and alkylate as normal, acidify as normal and proceed as normal. You can also use the high recovery protocol if you believe the amount of protein is low. Note that you must use very high concentrations of alkylating reagents if proteins were eluted in 5% beta-mercaptoethanol (BME): neat BME is 14.21 M making 5% BME 710 mM! Avoid this if possible.

Can I use S-Trap micro column for < 1 ug protein e.g. 50 ng of total protein?
Yes. The S-Trap micro column is compatible with low amounts of sample especially when used with the high recovery protocol available on our website. The performance of sample preparation depends especially on the complexity of your sample and also on sample handling post digestion. Small amounts of less complex sample — for example, a purified protein, or histone preparation, etc. — split the signal of your sample less and are thus more likely to be detected. Similarly, samples are more likely to "work" with minimal manipulation: lyophilize (do not speedvac) combine elutions, sonicate the resultant peptides in a buffer compatible with loading on your LCMS, and analyze immediately.

I' m doing iTRAQ or TMT. Do S-Traps work for that?
Yes. The standard protocol is designed for use with isobaric amine labels (i.e. iTRAQ and TMT).

Can I use S-Traps to clean up click-chemistry reactions?
Yes. Perform either CuACC or copper-free strain-promoted click-chemistry reactions. Reduce and alkylate, then perform the normal S-Trap protocol.

I' m analyzing urine. What should I do?
First concentrate the urine either by lyophilization or a centrifugal filter to around 1 – 2 mg/mL then follow the standard S-Trap procedure including the addition of SDS to 5% to begin, acidification and addition of binding buffer. Urine is usually around .08 mg/mL so typically a 10 – 20x concentration is necessary. Conditions of pathology may however significantly alter the protein concentration; determine protein content before processing.

I' m analyzing CSF. What should I do?
If your CSF samples are already aliquoted and frozen, lyophilize them. Bring them up in 8 M urea, 5% SDS, 50 mM TEAB pH 7.4 to approximately 1 – 2 mg/mL. (CSF is normally around 0.2 – 0.4 mg/mL so a 5 – 10x reduction in volume is typical.) Sonicate the samples (keeping the time of sonication identical), reduce and alkylate and process as per the standard protocol. Speed-vacing in the liquid state is to be avoided as it may introduce changes. If you have to aliquot the CSF samples, minimize freeze thaw.

I' m analyzing serum/plasma. What should I do?
Dilute the serum or plasma into 1X 5% SDS lysis buffer to a final concentration of 2 mg/mL. Serum and plasma are typically around 80 mg/mL so a typical dilution factor is 40x. Reduce and alkylate the proteins in this SDS solution, then proceed with the standard protocol including acidification, dilution with the binding buffer, application to the S-Trap, and cleaning and digestion in the trap.

I am analyzing peptidoglycans. What should I do?
Resuspend your sample in 0.5% Triton X-100 or Brij 25 with harsh ultrasonication until full cellular disruption: monitor by microscopy. Add lysozyme and incubate for 2 hrs at 37 C. Add SDS to 5% and ultrasonicate again, aiming for full dissolution of your sample. Perform a BCA assay, aliquot the desired amount of protein, reduce, alkylate and perform the standard S Trap protocol.

Can I use S-Trap protein extraction protocol for a gram-positive bacterial cell culture?
Yes. Use 5% SDS, optionally with cryopulverization, and ultrasonication. [Have hover-over for all instances of ultrasonication and cryopulverization.]

Can I use S-Trap protein extraction protocol for secreted proteins?
Yes. Take the solution containing your analyte molecules such as serum-free cell culture supernatant, add SDS, concentrate as needed by lyophilization. After concentration, perform a protein assay and run the desired S-Trap protocol.

Do you have a S-Trap protocol for fecal proteomics?
1) Extract feces with 5% SDS. You might want to extract metabolites etc. however beforehand with MeOH/acetone/ACN which probably will also clean up whatever proteins. Note that seed storage proteins, if any are left, are MeOH soluble. Spin out insoluble particulate.
2) Perform a BCA assay and aliquot the appropriate amount of protein.
3) Perform reduction/alkylation and the standard S-Trap protocol.
4) If MeOH insoluble pigments are left, wash with other organics including IPA, ACN, xylene, MTBE, and 50% MeOH/CHCl3. After any additional washes, "reset" the column by washing with standard S-Trap binding buffer. Ideally the column will look "clean".

Can I use the S-Trap to prepare samples (for gel analysis, etc.)?

Yes, it is completely possible to use S-Traps to clean up sample especially for SDS-PAGE gels. In this case, concentrate and clean your proteins in exactly the same way but do not add trypsin. Rather, add 1X SDS-PAGE buffer to the trap (the volume depends on the size of the wells in your gel; at least 20 uL however), heat the trap to solubilize the proteins (5 min at 95 C) and spin out your eluted proteins. If you have a lot of protein, this single elution is fine, and recovery is typically around 90%. If you have only a small amount of protein, then do three elutions with 0.5X Laemmli loading buffer, put it on a speed vac to concentrate it (the SDS will be a chunk then) and resuspend back to the right volume. It is a good idea to both reduce and alkylate proteins before purifying them with an S-Trap: first you don't have to worry about disulfide bond formation causing a ladder in your gel and because you can then go straight to Gel-LC if you want to do MS analysis.

Can I combine protein precipitation with S-Trap sample processing?

Yes. Perform precipitation — urine is especially popular — and resuspend the pellet in SDS. Perform a protein assay, reduce and alkylate, then process with S-Traps as normal.

I'm analyzing tissue. What should I do?

Please see the S-Trap protocol for mammalian samples.

What should I do with lipid rich samples?

The SDS lysis/solubilization buffer plus methanolic S-Trap washes are usually sufficient to take care of lipids from common samples like cell culture. However, like proteins, lipids have very disparate physiochemical properties and not all are methanol soluble. If you are working with a lipid rich tissue, such as brain or adipose tissue, you will probably want to do additional lipid cleanup.

There are two main approaches: remove the lipids before protein processing or remove the lipids when the proteins (and potentially methanol insoluble proteins) are on the trap. The best way to remove lipids before S-Trap processing is to use a cryogenic bead beater at liquid nitrogen temperature where the sample and an organic such as DCM, chloroform or ether and pulverized together. (Note at –196 °C, these solvents are solid. Typically, a 5 – 10-fold volume excess of organic solvent over tissue is used.) This sample is then warmed to 4 °C, vortexed, the organic removed (filtration or centrifugation; be cautious with the density of chloroform and DCM as proteins float) and the samples dissolved in 1X 5% SDS with harsh sonication (probe) or Covaris AFA. Proteins tend to be denatured by organic lipid extractions and require extra encouragement to go into solution.

To remove lipids on the trap, follow the S-Trap protocol up to the binding and first wash step. Then, rinse the proteins with an appropriate solvent (see below table). We recommend 2:1 chloroform/methanol to begin. Do the next two washes with the 90% methanol S-Trap binding/wash buffer and proceed with the protocol.

Solvent mixture(s); all v/v	Citation
10:3:2.5 MTBE/methanol/water; 60:30:4.5 chloroform/methanol/water	Cai, T., Shu, Q., Liu, P., Niu, L., Guo, X., Ding, X., ⋯ & Wu, P. (2016). Characterization and relative quantification of phospholipids based on methylation and stable isotopic labeling. Journal of Lipid Research, 57(3), 388-397.
30:25:41.5:3.5 chloroform/isopropanol/methanol/water	Shiva, S., Enninful, R., Roth, M. R., Tamura, P., Jagadish, K., & Welti, R. (2018). An efficient modified method for plant leaf lipid extraction results in improved recovery of phosphatidic acid. Plant methods, 14(1), 14.
10:3:2.5 MTBE/MeOH/water	Matyash V, Liebisch G, Kurzchalia TV, Shevchenko A, Schwudke D (2008) Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. J Lipid Res 49: 1137-1146.
2:1 chloroform/methanol	Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. The Journal of Biological Chemistry 226: 497-509.
Knittelfelder, O. L., Weberhofer, B. P., Eichmann, T. O., Kohlwein, S. D., & Rechberger, G. N. (2014). A versatile ultra-high-performance LC-MS method for lipid profiling. Journal of Chromatography B, 951, 119-128.	
4:1 methanol/chloroform	Dawson, G. (2015). Measuring brain lipids. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1851(8), 1026-1039.
1:2 chloroform/methanol	Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917.
1:1 chloroform/methanol; others	Reis, A., Rudnitskaya, A., Blackburn, G. J., Fauzi, N. M., Pitt, A. R., & Spickett, C. M. (2013). A comparison of five lipid extraction solvent systems for lipidomic studies of human LDL. Journal of lipid research, jlr-M034330.
3:1 butanol/methanol	Löfgren, L., Forsberg, G. B., & Ståhlman, M. (2016). The BUME method: a new rapid and simple chloroform-free method for total lipid extraction of animal tissue. Scientific reports, 6, 27688.
Many	Christie, W. W., & Han, X. (2010). Lipid Analysis-Isolation, Separation, Identification and Lipidomic Analysis, 446 pages.

High Throughput Proteomics

Do you have any recommended collection plate for the 96-well plate?

Any low protein binding plate which holds >400 ul should work. Our own favorite is the WheatonAntiBIND plates because you can also do the washes into them (1 mL capacity):
https://media.wheaton.com/documents/WHEATON_AntiBIND-Tech-note-1.pdf.
Eppendorf and Corning have similar plates cf.
https://online-shop.eppendorf.us/US-en/Laboratory-Consumables-44512/Plates-44516/Protein-LoBind-Plates-PF-16857.html
or
https://www.thomassci.com/E-K/Microplates/96-Well-Plates/_/96-WELL-MICROPLATE?q=Low%20Binding.
Also see https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5302088/.

Can I use <100 ug of starting material on the current 96-well plate?

Not recommended. 100 ug is the recommended minimum.

Can I use the unused wells on a 96-well plate later?

Yes. Keep the wells covered with packing tape especially during the protein digestion step in a humidified incubator.

How can I do high throughput proteomics?

Use PIXUL sonicator.

Does the 96-well S-Trap plates work with KingFisher?

Yes. Apply the standard S-Trap protocol steps.

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