Tube gel digestion

Developed in the Saito Lab and adapted by Noelle Held. See also Lu and Zhu Mol Cel Proteomics 2005

Materials

Make everything in LCMS grade H2O, use only LCMS grade reagents * 1M Tris HCL, pH 7.5 * 40% Bis-acrylmide L 29:1 * Ambic 25 (25mM ammonium bicarbonate) * 1% Ammonium persulfate (10mg/1mL H2O) * Ethanol washed 0.5mL and 2mL tubes, one each per sample * TE buffer (10mM tris HCL, 1mM EDTA in water) * At least 25ug sample protein (can be less but more difficult) * gel fix solution () * gel destain solution () * 50/50 wash (50% ACN:50% 25mM ammonium bicarbonate) * LCMS grade acetonitrile * 10mM DTT in ambic 25 (make fresh solution) (1.55mg/mL) * Ambic 25 * 55mM Ioda in ambic 25 (9.3mg/mL) * TEMED (full concentration) * 1% ammonium persulfate (10mg/mL) * bis-acryl L 30% solution

Preparation

- Prepare premix on ice: 1 part Tris HCL, 3 parts 40% Bis-acryl L (typically 252uL Tris HCL, 736.5uL Bis-acryl L)
- Prepare fresh DTT solution (I do not trust previously frozen, but you can get away with it!)
- Decide amount of protein to digest (I typically do 100ug digestions)
- Fill out the following table:

Sample	Sample concentration	Amt sample to be digested	Volume sample to be digested	Premix	1% APS	TEMED	TE buffer	Final volume
				103uL	2uL	2uL	2uL	7uL | 3uL | | 200uL
				103uL	2uL	2uL	2uL	7uL | 3uL | | 200uL
				103uL	2uL	2uL	2uL	7uL | 3uL | | 200uL
				103uL	2uL	2uL	2uL	7uL | 3uL | | 200uL
				103uL	2uL	2uL	2uL	7uL | 3uL | | 200uL
				103uL	2uL	2uL	2uL	7uL | 3uL | | 200uL
				103uL	2uL	2uL	2uL	7uL | 3uL | | 200uL

Protocol

- Place sample in clean 0.5mL tube, then add TE buffer in volume recorded above (calculate such that final volume is 200uL)
- Add the Premix

- Add TEMED and then APS, vortex speed 3 and and mix by pipetting. Work quickly.
- Allow to polymerize in thermomixer 1hr at 20C with no shaking START:
- Add 200uL gel fix solution to gels, incubate RT 20min
- Remove liquid, transfer gels to 2mL tubes (use a 200mL pipette tip)
- fix in 1200-1600uL gel fix solution (enough to cover) at RT 350RPM for 1 hr. START:
- Remove liquid, add 1600uL destain solution and incubate 2hrs RT 350RPM. START:
- Remove liquid, decant gels onto a clean surface and cut into 1mm cubes with a clean scalpel or razor blade, return to tube
- Add 1mL 50:50 wash, shake 350RPM for 1hr Note: This is a good place to break, gels can remain in incubator over night if needed
- Remove first wash solution, repeat wash step
- Dehydrate gels by adding 0.8mL acetonitrile, vortex, incubate RT 10min and remove supernatant. Repeat 2x until gels are hard.
- Add 600uL DTT solution, mix 1hr 56C 350RPM
- Remove liquid, note how much was absorbed by the gels (to calculate hydrated gel volume)
- Add 600uL Ambic 25, vortex, and remove (rinse step)
- Add 600uL Ioda solution, incubate 1hr RT 350RPM
- Remove Ioda, add 1mL ambic, vortex, incubate 10min RT 350RPM and remove solution
- Dehydrate with 1mL ACN, 10min room temperature and remove solution, repeat 2x
- Meanwhile, resuspend 100ug trypsin in 1000uL ambic on ice, allow to sit at least 30 mins to dissolve fully
- The desired trypsin:protein ratio in the digestion solution is 1:20. Calculate the desired concentration of trypsin to reach this amount below:

Sample	ug protein used in digestion	ug Trypsin to equal 1:20 ratio	Volume of hydrated pellets (record from DTT addition)	Desired concentration of trypsin (=ug trypsin desired/volume of hydrated pellets)	Starting concentration of trypsin (typically 0.1ug/uL)	Volume starting trypsin solution (= desired conc * volume / starting conc)	Volume ambic to acheive desired concentration (= starting volume - volume starting trypsin solution)

- Add the desired volume of trypsin to each sample (see table above), incubate 37C for 20mins
- Freeze any extra trypsin in small aliquots to be used later
- Add minimal amount of Ambic 25 to ensure rehydrated gels are covered with liquid
- Vortex, briefly spin down and incubate 37C 350RPM overnight. Check pH, should be about 8. START:
- END of digestion period:
- Spin down, collect liquid supernatant into clean 1.5mL tubes
- Add 50uL peptide extraction buffer, incubate 20mins RT
- Spin down and collect suspension, combine with liquid supernatant from above
- Repeat extraction step, combining the suspensions

- Spin the samples 20mins at high speed to remove any remaining debris
- Collect the top 90% off the centrifuged samples and concentrate on speed vac on low (as needed). Note the volumes and calculate the final estimated concentration of protein. Typically we seek 1ug/uL mixtures that are diluted to 0.1ug/uL in Buffer B and acidified to pH 4 for LC-MS/MS analysis

Sample	ug protein digested	Volume of sample after concentrating	Final concentration