Tube gel digestion

Developed in the Saito Lab by Vladimir Bulygin and Dawn Moran; adapted here 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)

Preparation

- Day 1: Prepare premix on ice: 1 part Tris HCL, 3 parts 40% Bis-acryl L (typically 252uL Tris HCL, 736.5uL Bis-acryl L)
- Day 2: Prepare fresh DTT solution (I do not trust previously frozen, but you can get away with it!) You will need about 600ul per sample at 1.55mg/mL in ambic 25. Prepare also IODA solution if needed.
- Decide amount of protein to digest (I typically do 100ug digestions)
- Fill out the following table:

Sample	Sample conc	Vol to Digest	Premix	TEMED	APS	TE	Final Vol
			103	3	7		200
			103	3	7		200
			103	3	7		200
			103	3	7		200
			103	3	7		200
			103	3	7		200
			103	3	7		200

Protocol

- Place sample in clean 0.5mL tube, then add TE buffer in volume recorded above (calculate such that final volume is 200uL)
- Add 103uL Premix to each sample
- Add 3uL TEMED and then 7uL APS and vortex. Work quickly. I usually add TEMED to all samples, then vortex, then add APS one by one vortexing each as I go.
- 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 to unstick the gels from the

tubes)

- 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. This can be shortened to 1hr with no negative effects that I am aware of. 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 50:50 wash solution, repeat wash step
- Dehydrate gels by adding 0.8mL acetonitrile, vortex, incubate RT 10min and remove supernatant. Repeat 2x until gels pieces are hard and white.
- 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 0.8mL 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 OR use previously reconstituted trypsin.
- 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	ug trypsin 1:20	volume pellet	ug trypsin/ vol pellet	vol trypsin	vol ambic

- Add the desired volume of trypsin and additional ambic 25 to each sample (see table above), incubate 37C for 20mins
- If new trypsin was reconstituted, 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

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