Triton-Acid-Urea (TAU) Gel Electrophoresis of Acetylated histones

Note: The gel described contains 15% acrylamide, 9mM Triton X-100 and 8M Urea. Gradient gels usually vary the concentration of Triton-X100.

First part of the protocol is based on Waterborg JH (**2002**) Acid-Urea-Triton polyacrylamide gel electrophoresis of histones in *The Protein Protocols Handbook*, 2nd Ed. The blotting procedure is based on Delcuve & Davie (**2002**) Protein blotting of basic proteins resolved on Acid-Urea-Triton-polyacrylamide gels, in *The Protein Protocols Handbook*, 2nd Ed.

Step 1 – *Assemble, Pour & Run the Gel*

Materials & Buffers: NB many are made fresh on day – they can't be stored

Acrylamide (60% w/v in ddH_2O) (Do NOT heat to get into solution – it takes <1 hour)
bis-acrylamide (2.5% w/v in ddH_2O) (Do NOT heat to get into solution – it takes <1 hour)
Glacial Acetic Acid (17.5M)
Concentrated NH_4OH (28-30%, ≈ 15M)
Triton X-100 (25% in ddH_2O, ≈ 0.4M)
TEMED
Riboflavin 5'phosphate (0.006% in ddH_2O; 3mg/50ml): Make fresh @ 10X stock; it's cheap
Phenolphtalein indicator solution (1% w/v in 95% EtOH: 1ml tubes @ -20ºC)
Methylene Blue (2% w/v in sample buffer)
Urea (powder)
Urea (8M): 12g to 25ml with ddH_2O. Add 1g Mixed bed resin (BioRad AG501-X8). Pipette 1.2ml aliquots to ependorff tubes and store @ -20ºC.

TAU Electrophoresis buffer (stable indefinitely @ RTº - store as 5X Stock)
1M Acetic Acid (17.5M Stock = 57.15ml/L; 5x = 285.75ml/L)
0.1M Glycine (mw 75.07 = 7.5g/L; 5x = 37.5g/L)

Protocol:

1. Use the Hoefer large gel – this will give the desired separation / resolution. Clean thoroughly and assemble the gel plates. Place in the apparatus and confirm a tight seal to avoid leakage.

2. Make the *TAU resolving gel* (fresh before use – do not store)

17.5ml	60% acrylamide
2.9ml	2.5% bis-acrylamide
4.2ml	Glacial Acetic acid
230µl	conc. NH_4OH
33.6g	Urea

Make to 63.5ml with ddH_2O. Stir to get Urea into solution. Confirm vol. Transfer to a 200ml sidearm flask with magnetic stirbar. While stirring vigorously apply a vacuum for 10 secs to degas. Add …

1.575ml	25% Triton
350µl	TEMED
4.67ml	0.006% Riboflavin 5'phosphate

3. Mix well and pour between the assembled plates leaving space for a stack gel. Carefully pipette a ddH_2O overlay to obtain a flat separating gel.

4. Switch on the lightbox and stand it horizontally as close as possible to the assembled gel stand. Place a sheet of aluminum foil behind the plates, reflective surface facing inwards, to increase light intensity and homogeneity. Gel polymerization should be detectable within about 2 minutes, and complete in **30 minutes** (the yellow color will fade as it polymerizes). Switch off the lightbox and remove the foil. Remove the ddH_2O overlay, rinse with ddH_2O, and wick out residual liquid with a sheet of 3MM.

5. Make the *TAU stacking gel* (fresh while resolving is polymerizing)

1.34ml	60% acrylamide
1.28ml	2.5% bis-acrylamide
1.14ml	Glacial Acetic acid
70µl	conc. NH_4OH
9.6g	Urea

Make to 18.6ml with ddH_2O in a 50ml Falcon. Place on rotator to solubilize Urea. Confirm vol. Transfer to a 50ml sidearm flask with magnetic stirbar. While stirring vigorously apply a vacuum for 10 secs to degas. Add …

100µl	TEMED
1.3ml	0.006% Riboflavin 5'phosphate

6. Mix well and pour between the assembled plates. Carefully insert the 15-tooth comb and ensure there are no air bubbles. Switch on the lightbox and stand it horizontally as close as possible to the assembled gel stand. Place a sheet of aluminum foil behind the plates, reflective surface facing inwards, to increase light intensity and homogeneity. After **60 minutes** switch off the lightbox and remove the foil.

7. Meanwhile make 1ml *Sample buffer* (should be intensely pink)

7.7mg	DTT (1M final)
900µl	8M Urea (from –20ºC, resolubilize and avoid the mixed bed resin)
50µl	Phenolphtalein (1% w/v in 95% EtOH)
50µl	30% NH_4OH

8. The preferred sample is a salt-free lyophilisate (Cl^- ions can interfere with the stacking). Note that the usual sample load per well is 50µl. It is probably best to store samples in aliquots sufficient for one or two lanes. Add 100µl sample buffer. To ensure full reduction of all proteins by DTT, the sample pH should be >8.0. If the pink color disappears because of residual acid in

the sample, add a few µl NH_4OH. Solubilize for 5 minutes at room temperature. Acidify with 5µl glacial acetic acid and add 4µl Methylene blue dye (2% w/v in sample buffer).

9. Move the plates into the buffer tank. Carefully remove the comb and wash out the unpolymerized gel with *TAU electrophoresis buffer*. Fill the wells with buffer and carefully load the samples to the bottom with a fine pipette. Assemble the top tank and carefully fill with buffer. Ensure there are no leaks.

10. Plug in the power supply. **NOTE: REVERSE from SDS-PAGE: Red to Black**. For maximum resolution through a 1.5mm thick / 18cm wide gel, the initial current should not exceed 25mA (about 100V). For the large Hoefer with an 11cm resolving gel a complete run is ≈15h @ 100V constant voltage: electrophoresis is complete just before the methylene blue exits the gel.

Step 2 – *Transfer / probe the Gel*

Materials & Buffers:

Supported nitrocellulose transfer membrane (prewet >1h in *CAPS transfer buffer*)
Glacial Acetic Acid (17.5M)
SDS (10% in ddH_2O)
β-Mercaptoethanol
1M Tris.Cl pH 6.8
400mM CAPS (88.5g in 800ml, adjust pH to 10 with 10M NaOH, make to 1L)
Thimerosal (1%: 100x for antibodies, 1000x for Blotto)
PBST: PBS / 0.4% Tween
PBST-I: PBS / 0.3% Tween / 0.1% India ink (Pelikan Fount India, Black 17)
TBST: 1 x TBS, 0.1% Tween
Blotto: 1 x TBS, 0.1% Tween, 5% Milk powder + Thimerosal

10x TBS (1L)
- 200mM Tris.Cl pH 7.5 (1M stock = 200mls)
- 1.5M NaCl (5M Stock = 300mls)

Equilibration buffer 1 (200ml, made fresh)
- 50mM Acetic Acid (17.5M Stock = 571µl)
- 0.5% SDS (10% Stock = 10ml)
- H_2O to 200ml

Equilibration buffer 2 (100ml, made fresh)
- 62.5mM Tris.Cl pH 6.8 (1M Stock = 6.25ml)
- 2.3% SDS (10% Stock = 23ml)
- 5% β-Mercaptoethanol
- H_2O to 100ml

CAPS Transfer buffer (2L)

25mM CAPS pH 10 (400mM Stock = 125ml)
20% MeOH (100% Stock = 400ml)
H_2O to 2L

Protocol:

11. Disassemble the apparatus. Gently shake the gel in 100ml *Equilibration buffer 1* for **30 minutes**. Discard buffer and repeat this wash for **30 minutes**. Discard buffer and wash with *Equilibration buffer 2* for **30 minutes**.

12. Assemble a gel sandwich as for SDS-PAGE with everything pre-wetted in *CAPS Transfer buffer*. Place the gel holder in the tank with the gel facing the cathode **(Black, -)** and the membrane facing the anode **(Red, +)**. Fill with buffer and transfer in the cold room at 70V for 2h or 30V overnight.

13. Disassemble the sandwich and wash membrane with 3 x 10 minute changes TBS-T. From this point on everything is like a standard SDS-PAGE western. It is recommended to confirm transfer with India ink staining: wash the membrane for 20 minutes with PBST, stain with PBST-I for 20 mins. Destain with TBS-T. Block for one hour with Blotto. Western probe as normal.