3/07/2019

I want to run out samples from yesterday’s extraction and blot with anti-gamma-H2A.X. Control? Have samples from 6/01/2018 (Col 20 and Col 60 min). 8 ug was

Poured **two 15% minigels** on 3/6/2019. Store at 4 degrees. 1mm wide, 10 well ea.

**Run out 8 ug of each sample**

1. 5 ul PageRuler

2. sample 1, Col, no IR

3. sample 2, Col, 20’ post IR

4. sample 3, Col, 60’ post IR

5. sample 4, Col, 120’ post IR

6. sample 5, xct-2, no IR

7. sample 6, xct-2, 20’ post IR

8. sample 7, xct-2, 60’ post IR

9. sample 8, xct-2, 120’ post IR

10. *positive control: Col, XX’ post IR from 6/1/2018 extraction*

Electrophoresis and blotting

1. **Don’t heat** urea-containing samples above 37 degrees before loading on gel! Instead, just let sit at room temperature for a few minutes before loading.
2. Marker is PageRuler prestained ladder (Thermo). Use 5 ul.
3. Start running at 150 V, then switched to 200 V when in separating portion of gel. Let blue dye run off the gel.
4. Transfer: soak gel for 30 minutes in equilibration buffer (Tris, 6.8; SDS, beta-ME). (two changes of buffer)
   1. 10 min in equilibration buffer x 2
   2. 5 min in CAPS buffer.
5. transfer in CAPS buffer to 0.45 micron PVDF (Immobilon – wet in methanol!). Pre-chilled buffer to 4 degrees for ~2 hrs.
6. Transfer for 2 hr at 70 V/ 300 mA at room temperature. Added a little ice pack to the transfer cassette. Started at 4:30 pm.
   1. (*alternatively: transfer for 16 hr at 65 mA (10 V?) in cold room,*).
7. Stopped transfer at 6:30 pm. Rinsed blot in TBS-T. cut off upper right corner of blot. Then blocked in 5% milk (Carnation, opened in 2017) in TBS-T at room temperature from 6:30 – 7:30 pm.
8. Rinse blots in TBS-T briefly 3 times.
9. Incubate in Sigma anti-gamma H2AX antibody (Sigma product # H5912, batch # 046M4791V ) diluted 1:20,000 in TBS-T. 4 degrees overnight, from \_\_7:45 pm \_\_\_\_\_\_\_\_ (*20 ml total volume*)
10. . \_\_\_\_\_\_\_ am. Rinse blots in TBS-T several times. Incubate in Invitrogen anti-rabbit-HRP secondary Ab at 1:20,000 (recommended range is 1:2,000 – 1:20,000; Invitrogen, A16096) for one hour. Room temperature, \_\_\_\_\_\_\_\_\_\_- pm – XX . Wash 3x with TBS-T, 10 min each. Develop. Worked well but a bit blotchy (sloppy transfer?).
11. I will rinse well in TBS-T, and then incubate in mouse anti-actin (1:20,000) for 1 hr, from \_\_\_\_\_\_\_\_\_\_\_\_ pm to \_\_\_\_\_\_\_\_\_\_\_ pm.
12. Rinse briefly 3x. Incubate in anti-mouse –HRP at 1:20,000. Room temperature, 5:20 pm – 6:15 pm.
13. Wash 3 x 10 min in TBS-T.

3/8/2019

I think I had poor transfer of lanes 3 and 4. I cut off the last lane (positive control). Overall, unimpressive.

Next: don’t bother with anti-actin blot; protein too big (about 42 kDa or so)

Run new gel: this time, dilute protein samples so all should be about the same concentration. Then do Bradform to confirm. Then run gel. Cut new PVDF membrane next time.

Load more protein next time.

🡪 look for better centrifuge tubes for next time, too.

Possible rotors: Beckman SW28 or 80Ti.

Beckman JA-20.1 (480 ml)

Nominal tube dimensions (largest tube) . . . . . . . . . . . . . . . . . . 18 × 99 mm Nominal tube capacity (largest tube) . . . . . . . . . . . . . . . . . . . . . . . . 15 mL

(maximum fill volume = 12.5 ml)

This would work.

Possible tubes:

342080 $240 for 100 of them

342081 $179 for 100 tubes

342082 $179 for 100 tubes

Swinging bucket?

SW28 = 38 ml. too big.