One-dimensional SDS-PAGE (9-18% TGX gel)

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

Protocol for one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 9-18% TGX gel for visualization and quantification of histone proteins.

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Sample preparation

1 Dry samples (equal to 400.000 cells)

2 Resuspend samples in 10µl laemmli-buffer

3 Add 1µl β-mercaptoethanol to each sample

Safety information

Perform this step in a fume hood

4 Vortex and spin down

5 Incubate for 7 minutes at 95°C in a thermoshaker

6 Spin down

Prepare Criterion Cell

7 Place the criterion cell on ice in a fume hood

8 Remove the sticker from the bottom of the gel cassette and check the gel for cracks

9 Put the gel cassette in the criterion cell

10 Fill the reservoir with running buffer (25mM Tris, 0.1% SDS, and 192mM glycine in MilliQ water) and take out the comb

Running of the samples

11 Load the samples and standards (2 µg of bovine histones) on the gel (3 standards per gel: lane 1, lane 9 and lane 18)

12 Put the cover on the criterion cell

13 Start running the gel on 200V

14 Stop running when the frontline is almost gone

Visualization

15 Take out the cassette

16 Incubate in fixation-solution (7% acetic acid, 10% methanol in MilliQ water) for 10 minutes on a shaker

17 Wash the gel 3 times for 5 minutes in MilliQ water on a shaker

18 Incubate in SyproRuby overnight

Overnight

19 Wash the gel 3x for 10 minutes in MilliQ water on a shaker

20 Visualize the gel (Versadoc)