Fluorescence gel of cell lysates

* Plate U2OS cells at 1e6/well into a 6-well plate.
* Stain cells overnight with desired Halo and/or SNAP ligands at 50 nM each.
* Rinse once with 1x PBS.
* Trypsinize cells, resuspend in 1 ml of DMEM, and count on the Countess.
* Spin cells down in Eppendorf tubes for 2 min at 200 g.
* Either freeze at -80 or resuspend immediately in 200 µl of SDS loading buffer. It will get very gloopy, but try to resuspend as much as possible with a P1000.
* Pass through a 26-gauge needle 10-15x to make less viscous. This will be very difficult at first. Try to avoid bubbles as much as possible.
* Heat to 95 deg C for 5 min.
* Load the equivalent of 100,000 cells per well on a Bis-Tris gel. I like to use my custom 3D printed combs, which allow me to load more sample per well.

Conveniently, the same gel can be imaged on the Pharos imager and then transferred to do a Western blot. It typically works best to use the “low” signal setting on the Pharos imager.

# 1x SDS loading buffer

Following Cattoglio et al., 2020 Elife

Mixture without BME:

|  |  |
| --- | --- |
|  | Per 400 ml |
| 50 mM Tris-HCl, pH 6.8 | Add 2.4 g of Tris free base to < 300 ml water; adjust pH with HCl |
| 2% SDS | 8 g |
| 10% glycerol | 40 ml |

To 40 ml, add the following:

|  |  |
| --- | --- |
| 100 mM DTT | 0.62 g |
| 0.625% BME | 0.25 ml |

(If you’re making this for anything other than fluorescence imaging, add 0.2% (w/v) bromophenol blue.)

Note: I initially tried making a 4x solution, but the SDS does not stay in solution. Make 1x instead.

**Tips:**

* Select “low sample intensity” in the Cy3 and Cy5 channels on the Pharos imager for JF549 and JFX650, respectively.
* When casting the gel, leave ~2 cm at the top of the gel for the stacking gel. Pipette a thin layer of isopropanol over the resolving gel to make the interface flat, and remove this thoroughly before pouring the stacking gel.
* Rinse your wells out very well with 1x MOPS running buffer, using a syringe with a needle to remove any traces of polyacrylamide that could interfere with loading and distort your bands.
* The 3D printed combs are a bit sticky, and it may look like the wells are messed up when you first pull them out. They’re fine. Apply gentle pressure to the cassette and squeeze out any bubbles, and the wells will re-form in the correct shape.
* Also because the 3D printed combs are a bit sticky, you should clean the stacking gel off of them thoroughly right after removing them.

Examples:

A screenshot of a computer screen

Description automatically generated

A close-up of a dna test

Description automatically generated