

Date: _____

Preparing Tissue Homogenates for Western Blots

by Laura Bonfini & Chris Karlovich

Purpose/Description of experiment:

Prepare: 95°C heating block, boiling water bath, dry ice.

Eye discs:

1. Dissect eye discs in 1XPBS, and transfer into 1XSDS loading buffer prewarmed to 95°C. The # of discs you dissect, the volume you wish to load in the gel etc. depends on the size of the gel and the level at which your protein is expressed. Below are some examples that have worked for people in the lab:

<u># disc pairs</u>	<u>volume buffer</u>	<u>size gel</u>	<u>size comb</u>
25	30	medium	10-12 wells
50	30	medium	10-12 wells

2. Homogenize for about 1 minute.
3. Boil homogenate for 2 minutes.
4. Cool down homogenate on ice, then add 1/10 volume 1M DTT in 1X loading buffer.
5. Boil homogenate for 1 minute.
6. Spin down cuticle for 5 minutes.
7. Transfer supernatant to a fresh tube.
8. Freeze homogenate down to -70°C in dry ice/ethanol bath, and throw in -70°C freezer.