PROTEINSIMPLE WES PROTOCOL- 1% SDS

I. SAMPLE PREPARATION (CO_2 & MW)

1. Make up 1% SDS solution
    - % solution = (dry mass in grams / volume in mL) x 100
    - Include 1X cOmplete Mini – A 10X stock solution – 1 tablet in 1 mL 1% SDS is stable for 2 weeks at 2-8^{o}C, or for 3 months at -15 to -25^{o}C.
    - Include 1X PhosSTOP - A 10X stock solution - 1 tablet dissolved in 1 mL 1% SDS is stable for more than 1 month if stored at 2-8^{o}C or for at least 6 months at -15 to-25^{o}C.
2. Weigh tissue
3. Add 10 volumes (w/v) of HOT (90^{o}C) 1% SDS solution
4. Sonicate
    - Setting: 3
    - On ice
    - Burst of 10 seconds, touching tissue
    - Repeat another 2 times if necessary; no tissue pieces left
    - If needed additional burst can be done (if big tissue piece) but make sure not to overheat the sample.
    - Keep sample on ice after sonication until all samples are sonicated.
5. Incubate while shaking at 90^{o}C for 10 minutes
6. Incubate on ice for 10 minutes
7. Spin for 5 minutes at 4°C
8. Decant into new tube
9. Measure protein content- BCA Assay
    - Dilute samples 1:100 with water
    - Make master mix: 200 µL reagent A + 4 µL reagent B for each sample/standard
    - Plate: 200 µL master mix + 25 µL sample/standard
    - Cover with film and shake for 10 seconds
    - Incubate at 37^{o}C for 30 minutes
    - Read plate
10. Aliquot samples (10-20 µL) and freeze at -80^{o}C

II. SAMPLE PREPARATION

- Make your Wes template, if using 13-cap, only use rows 7-19. Reaction size will depend on how many targets you are trying to run and if you can multiplex.

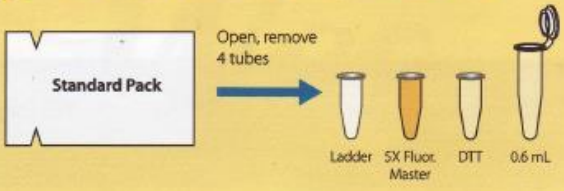
Capillary	Lysate	Stock Lystate Conc (µg/µl)	Lystate Conc (µg/µl)	Primary	Primary Conc	Secondary	Secondary Conc (µg/µl)	Reaction Size (µl)	Fluro Standard (µl)	Stock Lysate (µl)	0.1X Sample Buffer (µl)
1	Ladder										
2							neat		0	#DIV/0!	#DIV/0!
3							neat		0	#DIV/0!	#DIV/0!
4							neat		0	#DIV/0!	#DIV/0!
5							neat		0	#DIV/0!	#DIV/0!
6							neat		0	#DIV/0!	#DIV/0!
7							neat		0	#DIV/0!	#DIV/0!
8							neat		0	#DIV/0!	#DIV/0!
9							neat		0	#DIV/0!	#DIV/0!
10							neat		0	#DIV/0!	#DIV/0!
11							neat		0	#DIV/0!	#DIV/0!
12							neat		0	#DIV/0!	#DIV/0!
13							neat		0	#DIV/0!	#DIV/0!
14							neat		0	#DIV/0!	#DIV/0!
15							neat		0	#DIV/0!	#DIV/0!
16							neat		0	#DIV/0!	#DIV/0!
17							neat		0	#DIV/0!	#DIV/0!
18							neat		0	#DIV/0!	#DIV/0!
19							neat		0	#DIV/0!	#DIV/0!
20							neat		0	#DIV/0!	#DIV/0!
21							neat		0	#DIV/0!	#DIV/0!
22							neat		0	#DIV/0!	#DIV/0!
23							neat		0	#DIV/0!	#DIV/0!
24							neat		0	#DIV/0!	#DIV/0!
25							neat		0	#DIV/0!	#DIV/0!

- Thaw samples (single aliquot) at room temperature on day of use.
- Prepare the sample to your starting concentration. Example if sample is 15 µg/µl and want final concentration to be 0.5 µg/µl, best to make a 1 µg/µl stock to use for the run.
- The concentration of the "stock" will depend on the concentration needed in the Wes run.
- Likewise, the volume of "stock" needs also depends on volume needs in Wes run.
- Dilute your sample in 1X sample buffer (same buffer used in Wes assay). Note: Be sure to make enough buffer to dilute your samples and prepare reactions from Wes run, therefore your Wes template needs to be made prior to "stock" sample preparation.
- Keep "stock" samples on ice or at 4°C , while proceeding.
- Example of table I use to calculate and track sample lysis, total protein, and stock protein concentrations. I keep this in the sample excel file as the Wes template.

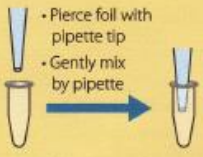
Sample	Weight	Buffer	Total Protein (µg/µl)			Sample	SB
		0.0		0	**0**	#DIV/0!	#DIV/0!
		0.0		0	**0**	#DIV/0!	#DIV/0!
		0.0		0	**0**	#DIV/0!	#DIV/0!
		0.0		0	**0**	#DIV/0!	#DIV/0!
		0.0		0	**0**	#DIV/0!	#DIV/0!
		0.0		0	**0**	#DIV/0!	#DIV/0!

III. WES REAGENT PREPARATION

A PREPARE STANDARD PACK REAGENTS



DTT (Clear Tube)

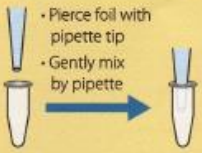


- Add 40 µL deionized water to make a 400 mM solution

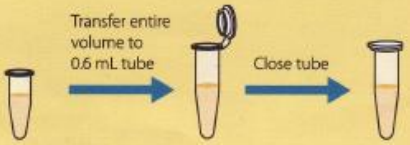
Fluorescent 5X Master Mix (Pink Tube)

- Add 20 µL 10X Sample buffer
- Add 20 µL prepared 400 mM DTT solution

Biotinylated Ladder (White Tube)

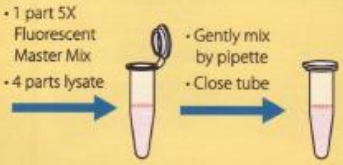


- Add 16 µL deionized water
- Add 2 µL 10X Sample Buffer
- Add 2 µL prepared 400 mM DTT solution



B PREPARE YOUR SAMPLES

- When using lyates, we recommend a final lysate concentration of 0.2 mg/mL. The optimal protein concentration depends on the expression level of your protein. If needed, dilute your lysate with 0.1X Sample Buffer (dilute 10X Sample Buffer 1:100 with water).
- Combine 1 part (e.g. 1 µL) 5X Fluorescent Master Mix with 4 parts (e.g. 4 µL) lysate in a microcentrifuge tube (final concentration 0.2 mg/mL).



C DENATURE YOUR SAMPLES AND BIOTINYLATED LADDER

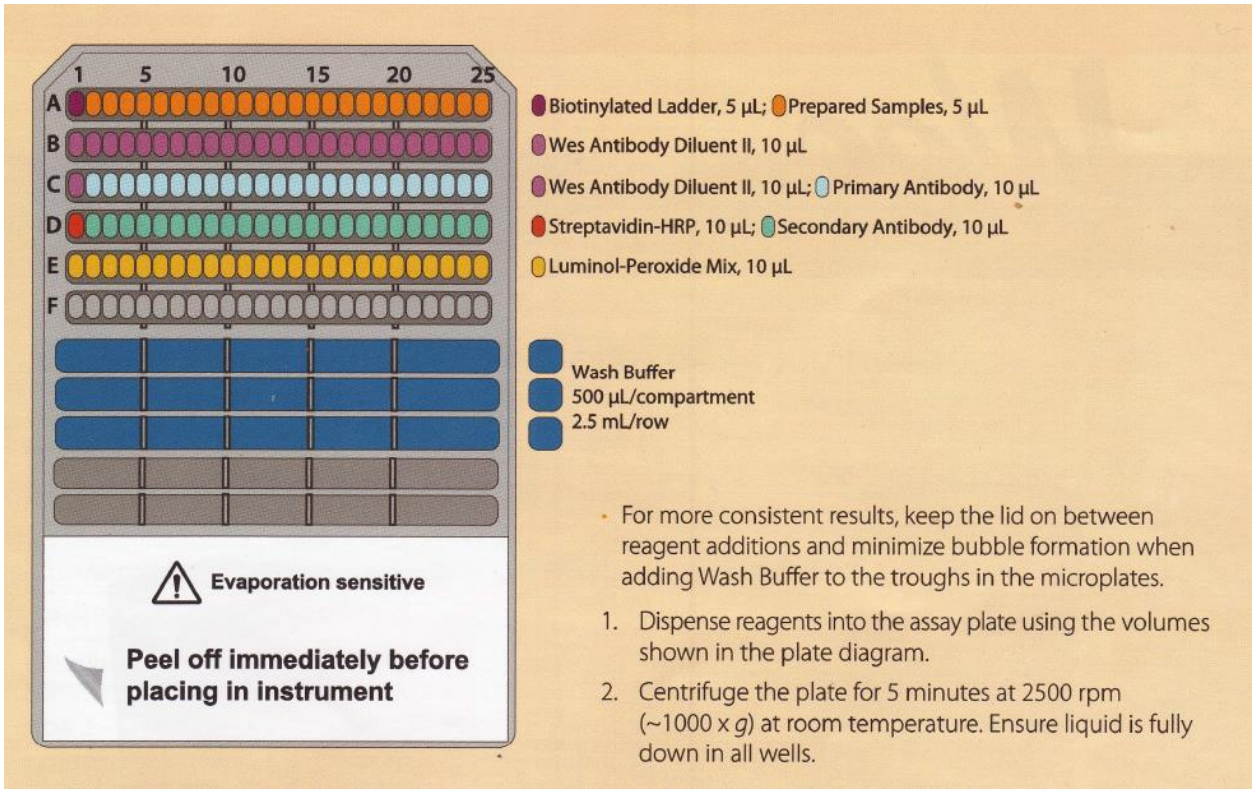
D PREPARE YOUR ANTIBODIES

- Primary Antibody: Start with a 1:50 dilution in Antibody Diluent II. The optimal antibody concentration can be titrated later, if necessary. If you're using the System Control, add 25 µL of the System Control Primary Antibody to 225 µL of your diluted Primary Antibody. Pipette 10 µL of the mixture into each Primary Antibody well.
- Secondary Antibody: The supplied antibody is ready to use without dilution. If you are using your own, consult your FAS or the Antibody Database on the ProteinSimple website.

E MIX LUMINOL-S AND PEROXIDE

- Combine 150 µL Luminol-S and 150 µL Peroxide in a microcentrifuge tube

IV. WES PLATING



Notes:

- **For 13 capillary plate use plate cover that covers un-used wells.**
- **Always balance plated before centrifugation.**
- **Never centrifuge at cool/cold temperatures.**
- **Cover Wes plate with full cover**

V. STARTING WES