University of Michigan Comprehensive Cancer Center	SOP-BCR-7.2	**Western Blotting for BRCA1 (D-9 Santa Cruz)**	Author: S. Clouthier Approved: M. Wicha	Rev: 1.0	Issued: 09/24/98 Revised: 7/3/12

1.0 Purpose

The purpose of SOP 7.2 is to provide details on how to prepare Fresh MC1 cells (from a paralyzed control mouse) for flow cytometry.

2.0 Scope

SOP 7.2 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

No.	Name	Description	Storage Location
1.0	1x PBS	Phosphate Buffered Saline	Cold Storage (026-380C)
2.0	Bio-Rad Protein Assay	Dye binding Assay	Cold Storage/Fridge #1 (026-380C/026-328S-A)
3.0	NER Buffer	Nucleotide excision repair Buffer	
4.0	CH_3OH	Methanol	Chemical Cabinet (026-314S)

4.0 Procedure

4.1 Thaw sample and stand proteins.
4.2 Put standard proteins (conc. Ranges from high to low [7 standards] and then water only) in first 3 columns of wells.
- Use 96 well plate

4.3 Vortex each protein before adding. Add enough protein + water to equal 10 µL. Place in 3 wells next to standard proteins. (Next vertical column of 3 wells)
4.4 Dilute Bio-Rad Protein Assay.
- One part Bio-Rad Protein Assay and 4 parts H_20.
  - 4.4..1 Mix by flipping 50 mL tube.

4.5 Add Bio-Rad Protein Assay mix.
- If concentration of protein is high, the color will be blue.

4.6 Put plate on the rocker for a few minutes.
4.7 Place plate in micro-plate reader.
- On computer select SoftMax (on desktop)
- Click Assay and then choose Bradford.
- Copy grid into Excel and save to jump drive to print elsewhere.
- On printout, calculate amount of protein to be used by sing average concentration.
  - 4.7..1 If you used 2 µL of protein to 8 µL of H_20 in step 3, multiply by 5 to get the final concentration (ng/µL).
- Calculate how many µL needed to get 80 µg.

4.8 Add protein sample blue buffer (6x) into new labeled tubes (#1-12).
- Vortex protein before adding.
- To make 60 µg of **nuclear protein**, based on concentration of protein calculate how much to add. If the protein is too high, add **NER Buffer** to dilute to 30 µL final; if the protein concentration is too low, go to dry the protein to make around 30 µL/30µg protein. No more than 40 µL total (protein + 6 µL blue dye).

- Put the gels into the tank and start to run the gel.

4.9 Boil in dish on hotplate for 5 minutes.

- While boiling, wash wells in gel running tank with syringes.
- Add markers to outside wells.
- Not numbered in tank.

4.10 After boiling, put on **ICE** while taking tubes to be spun down. Spin tubes briefly.

4.11 Load the samples into corresponding wells (from numbered tubes) and set timer for 1 to 1.5 hours.

- Check tank after 1 hour.
- Prepare membrane after 1 hour.
- Cut it, wash it in methanol for 20s and rinse in dHs0 for 5 minutes on rocker.
- Dump the dH20 and replace with transfer buffer: for high size protein (>100 kDa)= low concentration methanol (10%)
- Put membrane in buffer on rocker until gel is ready.

4.12 Set up transfer machine.

5.0 Applicable References

6.0 Change Description

Revision	Date	Reference	Description of Change
1.0	7/3/12	CL	Updated room locations