SOP-P045

Cervical Cancer Cells Preparation

Objective: To prepare cancer cells for blocking, staining, and sorting

Procedure:

1. When samples arrive, log each tube in on the provided inventory sheet. (scratch/projects/cervical cancer/(latest date) AAB cervical cell inventory) Then place them in the cold room for temporary storage.

2. Once the samples have settled after shipping, remove each sample carefully from the tray in which they came in. Try not to disturb the cell pellet and remove an appropriate amount of supernatant so the cells are more concentrated for counting.

3. Filter samples through 100um filter and transfer into 15ml cc tubes. (Millipore device, nylon net filters cat# NY1H02500, 100um NY1H) or Millipore Steriflip Vacuum-driven Filtration System Sterile. Disposable 50cc tubes. Cat# SCNY00060 (60um Nylon Net Filter / Qty: 25/pk)

4. **Cell Counts:** Take a 20ul sample from a tube and place it in a Cuvette with 10mls of PBS. Swirl gently and place on the Coulter Counter to check cell count. Range set for everything above 4um size. (Follow Coulter Counter SOP-P052 for operating instructions.) Then log in the cell count for each sample on the inventory list.
eg: (# x 10^6/ml)

Procedure: This is a 3 day procedure.

Day 1: Blocking (Only choose samples that have all ready been filtered and counted from the inventory list.)

From the counted samples, you will need to calculate the how many uls it will take to make a (l x 10^6/ml) concentration. Divide 1000(uls) by the cell count (7.436/ml) = 135uls needed from this sample.

eg: If the cell count was 7.436/ml, you will need 135uls to make 1x 10^6/ml

1. Once you have figured out the number of uls you need, pipette that amount into a 1.5ml eppendorf tube.
Do this for every sample you want to run.

2. Centrifuge these samples for 6 mins, at 25°C, @ *rcf 300. This is to remove as much of the PreserveCyte as possible.

3. 1st Wash - add 500uls of Buffer #1 (see SOP-P068 Buffers), let sample sit for 10mins in the dark at room temperature.

4. After the 10mins, centrifuge for 6 mins, at 25°C, @ *rcf 300. . Remove supernatant.

5. 2nd Wash - add 500uls of Buffer #1 (see SOP-P068 Buffers), let sample sit for 10mins in the dark at room temperature.

6. After the 10mins, centrifuge for 6 mins, at 25°C, @ *rcf 300. . Remove supernatant.

7. Samples are now ready for Blocking. Add 500uls of Buffer#2 (see SOP-P068 Buffers) and mix well by pipetting up and down several times. Leave overnight in the dark at room temperature.

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Day 2: Staining

Staining Cocktail:

p16^{INK4a}LL-PE: 4ug ÷ 1.25ug/ml = 3.2 x # of samples (10) + 1 for carry over
eg: 3.2 x 11 tubes = 35.2uls of p16^{INK4a}LL-PE

MCM5 LL-Cy5: 6ug ÷ 0.83ug/ml = 7.23 x # of samples (10) + 1 for carry over
eg: 7.23 x 11 tubes = 79.53uls of MCM5 LL-Cy5

Buffer #3: To Calculate the volume you will need to first add 3.2uls (p16) to 7.23uls (MCM5) = 10.43uls. Subtract the 10.43uls from 60uls = 49.57uls (Buffer #3) This is the volume need for 1 sample (60uls)

Now take 49.57uls of Buffer #3 x # of samples (10) + 1 for carry over
eg: 49.57 x 11 tubes = 545.27ul of Buffer #3.

Add the 35.2uls of p16^{INK4a}LL-PE and the 79.53uls of MCM5 LL-Cy5 and the 545.27uls of Buffer #3.
The total volume is 660uls of Staining Cocktail which is enough for 11 samples

1. Now take the samples that are in blocking Buffer #2 and centrifuge samples for 6 mins, at 25°C, at 300*rcf. Remove supernatant.

2. For the **stained** samples, add 60uls of the staining cocktail to the cell pellets. To the **unstained** samples, add 60uls of Buffer #3 to the cell pellets. Mix well by pipetting up and down several times.

3. Stain overnight in the dark.

Day 3: Washes, Ready to run on MoFlo XDP:

1. Add 500uls of Buffer #3 to each sample. Mix well by pipetting up and down several times.

2. Centrifuge for 6 mins, at 25°C, @ *rcf 300. Remove supernatant.

3. 1st Wash - Add 500uls of Buffer #4. Let sit for 10mins in the dark at room temperature.

4. Centrifuge, same settings, remove supernatant.

5. 2nd Wash - Add 500uls of Buffer #4. Let sit for 10mins in the dark at room temperature.

6. Centrifuge, same settings, remove supernatant.

7. Resuspend cells in 500uls of Clean PBS. Samples are ready to run on the MoFlo XDP Sorter.

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Verified by:_____Date:___9/5/2012 _____

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