

SOP-E026

Operation Procedure for Coulter Counter Model Z2

Objective: To determine the concentration of blood/cultured cells in an aqueous suspension.

Sample dilutions :

For most cell counts, and white blood cells (WBC's) a 1:500 dilution is made. Pipet 10 ml PBS (refer to SOP#-P020 for preparation of Phosphate Buffered Saline) into a disposable blood dilution vial ("Coulter vial"). Make sure your sample is well mixed before pulling up 20 μ l of cell suspension using a Pipetman. Quickly and carefully wipe off the outside of the pipet tip with a Kimwipe. Be careful not to hold the Kimwipe against the end of the tip, or the contents will be pulled out of the tip. Dispense this volume of cell suspension into the Coulter vial containing the 10 ml of PBS. Cap the vial and mix gently by inversion. Do not introduce air bubbles. The sample is ready to be counted. However.....

If the cell suspension is from a whole blood preparation, just before performing the count, add 3 drops of *ZAP-O-globin* or *Criterion Sciences Manual Lyse*, to lyse any remaining red blood cells that may be present. Cap the vial and mix gently by inversion. Don't introduce air bubbles. Allow to sit for 30 seconds for the lyse to work. The sample is ready for counting. Do not allow it to sit for more than 10 minutes, otherwise destruction of the WBC's will begin to occur. **NOTE: This step is not necessary if counting cultured cells where RBC's are not present.**

For red blood cells (RBC's), a 1:50,000 dilution of whole blood is made.

- a. Prepare a 1:500 dilution of the whole blood as above for the white blood cell count.
- b. Pipet 100 μ l of the 1:500 μ l dilution to 10ml of PBS in a counting vial. This is now a 1:50,000 dilution.
- c. Cap the vial and mix gently by inversion. The sample is ready for counting.

To load a sample:

To load a sample onto the coulter counter, press and hold the platform release catch and lower it down. Let go of the platform and it will remain at that position. Place the counting vial on the platform. Press and hold the release catch again, and gently raise it up until it stops. Be careful to ensure that the electrode is inside the counting vial and that it is not being hit or crushed. Let go of release catch.

Procedure:

1. Check the aperture size. If not the one you need see SOP-PXXX for instructions on how to change it.
2. Check the diluent jar to be sure it is full. Make sure you fill it with the proper diluent. ISOTONII, or homemade PBS may be in use. Check to be sure what is being used. If you must change diluent, the calibration procedure (SOP-PXXX) will have to be done before you can make a measurement.
3. Check the waste jar. If need to empty, unscrew the cap and dump the liquid down the sink flushing with lots of water.
4. Turn the power on. (toggle switch on front panel, top left).
5. At the data terminal, **ENTER SIZE DATA**

NOTE: The user should expect a specific population to have a limited range of values, therefore the size should be set accordingly.

- a) Press **SET-UP** on the data terminal until screen **S1: SETUP – ENTER SIZE DATA** displays:

S1: SETUP – ENTER SIZE DATA
100µM C, Kd=60
Select Units: <µm>
Set Upper Size Tu: 8.0µm
Set Lower Size Tl: 3.0µm
Count Mode: <above Tl>

- b) Check that the Aperture Tube and reference letter is the required one. (This can be altered on screen S2)
- c) Move to **Select Units** by using the cursor keys on the data terminal. Select either **µm** or **fl** (=mm³).
- d) Move to **Set Upper Size Tu**. Enter the upper size threshold you wish to measure. There is no upper limit to the numerical value of the size that may be entered, but the practical working limit is 60% of aperture diameter. The ratio of Upper to Lower size should not exceed 3:1 by diameter (27:1 by volume). If this ratio is exceeded, screen S9 will come up (S9: THRESHOLD RANGE TOO WIDE), when the START button is pressed.
- e) Move to **Set Lower Size Tl**. Enter the lower size threshold you wish to measure. The minimum size that can be measured is 2% of the aperture diameter.
- f) Move to **Count Mode**. You have the following choices:
- (1) **above Tl** – all particles that are equal to and exceed this setting are counted
 - (2) **between** – all particles that are equal to, or exceed the lower size setting, but do not exceed the upper size setting are counted
 - (3) **above Tu** – all particles that are equal to, and exceed this setting are counted

NOTE: all three count modes can be accessed at the end of an analysis.

- g) Press **SET-UP** and screen **S2** displays:

S2: SET-UP ENTER ANALYSIS DATA
Select Aperture: <100µm C>
Aperture Kd: 60
Metered Volume: <0.5>
Measure Aperture: <No>
Optimize Settings: <No>

- h) There are three situations here depending on the aperture tube/electrolyte solution combination:
- (1) **No change**: if the aperture tube and Kd do not need changed and the electrolyte solution is the same, move to **Metered Volume**, then select the appropriate volume for the aperture tube installed. Press **START** to initiate analysis
 - (2) **Change Aperture**: If the aperture tube selected is not the one that is fitted, move to **Select Aperture** and select the required one. Change **Measure Aperture** and **Optimize Settings** to **YES**. Move to **Metered Volume** and select the appropriate volume for the fitted aperture tube. Go to step (i.)

(3) **Change Electrolyte:** If the electrolyte solution type has changed, move to **Metered Volume** and select the appropriate volume for the fitted aperture. Then select **Yes** at **Measure Aperture** and **Optimize Settings**. Go to step (i.)

- i.) Press **START**, the counter automatically measures the aperture characteristics and determines the optimum instrument settings for the sizes entered. When complete, screen **S3** displays showing the recommended instrument settings. (screen **S7** functions the same as **S3**). Press **START** to accept the instrument settings and to initiate analysis.

6. **S3: SETUP – Instrument Settings Screen**

- a) Press **SET-UP** until screen **S3** displays:

S3: SETUP – INSTRUMENT SETTINGS
Avail. Range 2.569 – 9.7985 mm
Gain : 128 <128>
Current : 1.00 <1.00>ma
Preamp gain : 179.90
Tl = 13.3%, Tu = 22.9%

This screen allows the operator the opportunity to change the settings calculated by the instrument. In the unlikely case that changes are required, move to the **Gain** and/or **Current** fields and select the required values. NOTE: The left-most values for gain and current are the settings computed by the instrument's internal algorithm. The right-most values are the settings that the Z series will use. Initially these are set to the same value. If **Gain** and or **Current** are altered, the instrument may not be at its original size setting. If necessary, press **SET-UP** twice to return to screen **S1** to re-enter the required sizes(s).

7. Press **START** to initiate an analysis.
8. While the sample is counting observe the orifice for debris.
9. If something covers the orifice try the unblock button.
10. When it has analyzed the selected volume, it will stop counting and display the count.
11. Repeat the analysis two more times and record the counts.
12. If one count is way off, do another analysis and throw out the outlier.
13. Take an average of three counts.
14. To read the display:

For WBC Sample: Coulter Counter display reads 5400 counts
Count is 5.4×10^6

For RBC sample: Coulter Counter displays reads 5400 counts
Count is 5.4×10^8

NOTE: Accuracy is lost when the diluent level in the counting vial is not covering one third of the electrode. So if several additional counts are needed, you might have to make a new dilution.

Shutdown:

Prime the aperture using a non-lytic cleaner (Coulter Clenz), and leave the cleaning agent around the aperture overnight. Ensure the aperture and the electrode are submerged in the cleaning agent before setting the power switch to off.

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Date:

Verified by: _____ **Date:** _____

Print Name

Sign Name