

Procedure for Casy model TTC counter of ROCHE

AIM

To describe each step of preparation and counting the cells by Casy TTC. The protocol summarizes the complete customer's Training Guide given by supplier (Roche).

Material

Item	Reference	Supplier
Tube	5651794001	ROCHE
CASYTON	5651808001	ROCHE
CASYCLEAN	5651808002	ROCHE

Login : Installation

Password : casycasy

PC WINDOWS : Login : cih password : cih

Database:

casy.exe

cih/pasteurcih

Protocol

Turn on and clean the instrument :

(cf customer's Training Guide page 8 setting up)

1. Switch on the screen and the machine (one button behind and other on the right side of the system)
2. Perform Measure 1x CLEAN (F6) with casyclean
3. Perform Measure 1x CLEAN (F6) with casyton
4. Don not save (press cancel)
5. The counter is ready to count cells

Calibration :

(cf customer's Training Guide page 73 Preparing the Samples)

Note : Calibration enables identification of dead cells, viable cells and debris. Ensure the selection of appropriate protocol.

Note : Calibration is to be performed once per week .

Protocol to be selected for LabEx project (PBMCs and EBV cell lines):

« test 150 premiere » : 400µl – size 0-20µm – capillaire 150 – cursor 3,21/6,20

1. Take 200µl of diluted PBMCs (for example 10µl of PBMCs in 190µl of PBS), then add 800µl of CASYblue.

2. Wait 2 minutes and add 9ml CASYTON.
3. Count by clicking on M, adjust the position of cursor to eliminate dead cells.
4. If you change any parameter click on S to save the changes
5. Dilute 10µl of the diluted PBMCs without CASYblue, in 10ml of diluent buffer CASYTON.
6. Count by clicking on M in order to verify the position of viable cells, dead cells and debris.



Counting:

1. Dilute 10µl of the PBMCs of each sample in 10ml of diluent buffer CASYTON.
2. Count cells by clicking on M and verify the position of viable cells, dead cells and debris.
3. Write the number of cells counted and percentage of viability. If necessary, adjust the position of the cursor (Click on link « Analyse » - « Cursor » in order to unlock the settings) and repeat the counting.
4. Indicate the number/name of the sample (patient) Under « comment »
Note: If the sample is too concentrated, change its dilution in the « Analyse » tab

Note : Remember to use the aluminium cap while counting to protect from disturbance caused by neighboring electronic devices

Shut down :

1. Perform Measure 3x CLEAN (F6) with cleaning solution (casy clean)
2. Perform Measure 3x CLEAN (F6) with casyton
3. Verify the efficiency of cleaning by reading casyton (number of counts should be < 100)
4. If necessary replace the bottle of casy ton with casyclean (on the right side of the machine) and perform Measure 5x CLEAN (F6) with casy clean, followed by perform Measure 5x CLEAN (F6) with casyton.
5. Switch off the the screen and the machine

Stand by :

(cf Operator's Guide page 51 6.4.2 Dry liquid System)

Note : this protocol is to be used if the machine is unused during >3 weeks

1. Switch off the screen and the machine
2. Empty bottles
3. Take off the white external probe that aspirates sample
4. Rinse the probe in casyton and distilled water and store it in the white transparent box

Storage of files :

1. Save all data on the storagecard2. The card could record approximately 40 files.
Note : The counter generates two files for each read (.txt and .crt)
2. Unplug the storagecard2 and use the adaptor to plug the card to the computer.
3. Open the card named « Superstorage » and transfer the files into « CASY data » folder on the LabExMI Silo
4. Open casy.exe and verify on several downloaded files that they can be read.