

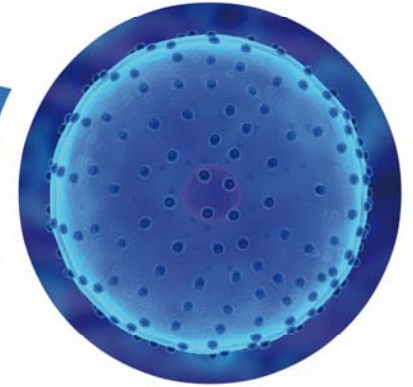


www.immunostep.com



STEM CELL KIT

Reagent kit for the quantitative determination of the Stem cells



PRINCIPLE

The SCK kit is designed to enumerate total and viable CD34 cells and to calculate their percentage as accurately, reproducibly, and rapidly as possible. It uses a tube with spheres of known number that allows us to estimate the cells using a single platform.

ADVANTAGES

1. It is a single tube assay that allows us to calculate simultaneously the percentage and the absolute number of cells per microliter of sample.
2. The kit allows us to identify non-viable cells and non-specific background signal.
3. Identification quick and easy way to double-positive CD45 / CD34.

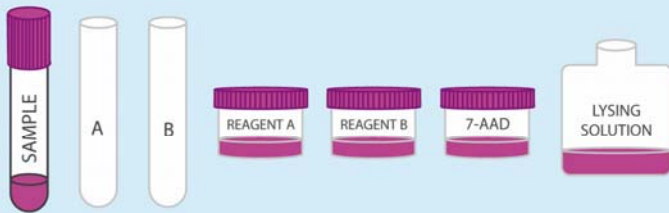
REAGENTS SUPPLIED WITH THE KIT

	SCK-25T	SCK-50T
Vial A	25 test CD45/CD34	50 test CD45/CD34
Vial B	25 test CD45/IgG1	50 test CD45/IgG1
Stepcount	50 test	100 test
7-AAD	50 test	100 test
10X NH4Cl Lysing Solution	5 ml	10 ml

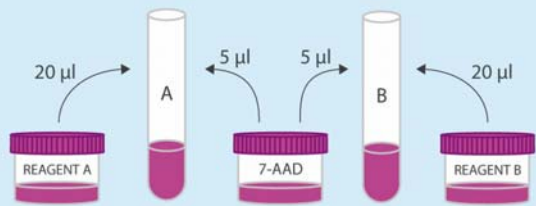
Required apparatus: Flow cytometer with 488nm.

ASSAY PROCEDURE

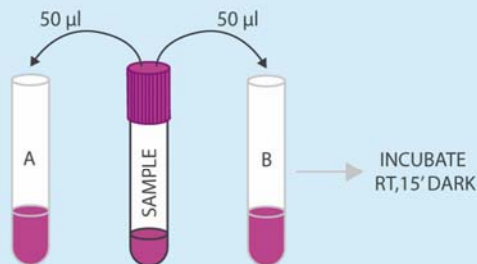
1. Sample and reagent preparation.



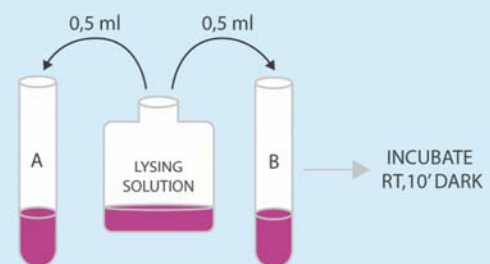
2. Labelling.



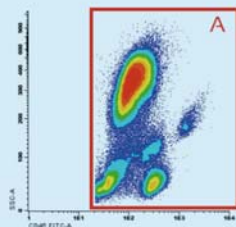
3. Adding sample.



4. Lysing



5. Adquisition

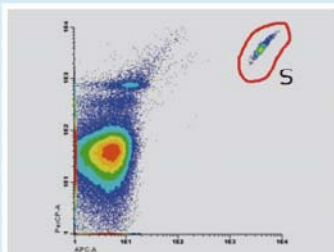


Set on the cytometer to store only the events in the region A.

Adquire and store all possible events.

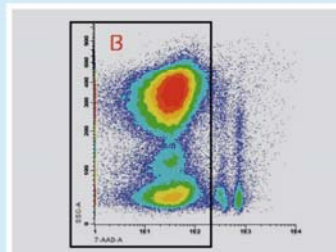
ANALYSIS EXAMPLE

Example of a fresh bone marrow (BM) specimen following analysis. Sample: 100 µl BM.

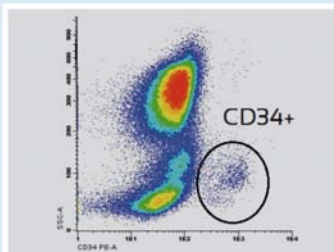


Step 1: Select the population of spheres.

If possible, remove the image to not interfere with the rest of the analysis.



Step 2: Select the viable cell population (region B)



Step 3: Using B Population selected CD34+.

Select the CD34+ cells in the tube A for specific CD34 cells.

Select the cells with the same gate in the tube B for determining nonspecific staining.

Results

Viable CD34 of total cells = 1,23% or 579 CD34/µl
 Total CD34 = 1,31% or 653 cells/µl
 Viable CD45 = 98,65% or 49250 CD45 cells/µl
 Total CD45 = 98,47% or 49125 cells/µl
 Viable CD34 of total CD34 = 91,96%
 Viable CD45 of total CD45 = 95,84%
 Viable CD34 of viable CD45 = 1,175 %

Step 4: To calculate the absolute count of cells.

The absolute number of the cell population of interest is determined by dividing the number of cells of interest acquired by the number of beads acquired (FL1/FL2), and multiplying this result by the microsphere concentration (microsphere concentration is indicated in the label on the tube).

CAUTION: 7-AAD is a potential carcinogen. It is recommended that the user wear protective clothing, gloves, and eye/face protection in order to avoid contact with skin and eyes.