Mouse IgG Calibrator

Kit for leukocyte surface antigen quantitation by flow cytometry

For 10 calibration curves and 50 tests **Ref. CP051**

Significant changes are indicated a dotted line in the margin

BioCytex

For Research Use Only.
Not for Use in Diagnostic Procedures.

1 INTENDED USE

Calibration kit for the measurement of human leukocyte surface antigen expression level by multi-color analysis.
Leukocytes are stained by indirect immunofluorescence with specific monoclonal antibodies (MAbs) and analyzed by quantitative flow cytometry.
Counter-staining reagents can be added to the procedure to gate and analyze sub-populations of interest. The expression level of the tested antigen is determined using the calibration beads.
The Mouse IgG Calibrator is designed for purified mouse MAbs of IgG1 and IgG2a isotypes.
The Mouse IgG Calibrator is also applicable on isolated cells.

2 REAGENTS

- **Reagent 1 :** 2 x 52 mL, diluent, 10 fold concentrated.
- **Reagent 2 :** 1 x 500 µL, calibration beads. The beads are coated with increasing and accurately known quantities of mouse IgG. The number of antigenic determinants coated on each bead population is indicated in the calibration flyer provided in the kit. The calibration values may slightly vary from lot to lot.
- **Reagent 3 :** 1 x 1.1 mL vial, staining reagent, polyclonal antibody anti-mouse IgG-FITC.
- **Reagent 4 :** 1 x 2.5 mL, neutralization solution.
- **Reagent 5 :** 2 x 11 mL, lysis solution, 10-fold concentrated.
- **Reagent 6 :** 1 x 30 mL, fixative solution.

The Mouse IgG Calibrator contains enough reagent to perform:
- 10 calibration curves
- 50 immunostainings (MAbs to be tested and corresponding negative isotypic controls).

3 REAGENT PREPARATION AND STORAGE

Intact kits and contents remain stable until the expiration date printed on the box label, when stored at 2-8°C.
After opening, the reagents are stable for 2 months when stored at 2-8°C and free of contaminations.
Do not freeze the kit.

- **Reagents 1 and 5 :**
  Prepare a **1:10 dilution** with distilled water.
  Prepare the appropriate volume required for the series to be tested.
  The presence of crystals does not affect the quality of the reagent 1.
  Incubate at 37°C until the crystals are completely dissolved.

- **Reagent 2:**
  After resuspension by vortex for 5 seconds, the reagent is ready for use.

- **Reagents 3, 4 and 6 :**
  Ready for use.

4 SPECIMEN COLLECTION AND TREATMENT

• Sample collection:
Use non-wettable (plastic or siliconized glass) blood collection tubes.

• Sample preparation
The test is performed on whole blood drawn on anticoagulant EDTA or Heparin.
Please note that quantifications can be noticeably different comparing EDTA and Heparin.
We recommend that each laboratory define its own expected values, from a local population of healthy donors covering the same age range as the tested patients for each anticoagulant.
Alternatively, the test can be performed on isolated cells the cell suspension must be adjusted at $2x10^6$ to $5x10^6$ cells/mL.

Sample storage:
- Blood must be preferentially stored at room temperature before testing (18-25°C). The sample stability is usually 24 hours at room temperature. However, it is recommended to check this stability for each tested parameter (antigen).
- Isolated cells (or cultured cells) must be stored according to their particular characteristics.

5 WARNING

- Follow the conventional laboratory practices.
- Blood must be considered as potentially infectious.
- Reagent 6 contains 2% paraformaldehyde :
  - **H317**: May cause an allergic skin reaction
  - **H319**: Causes serious eye irritation
  - **H341**: Suspected of causing genetic defects
  - **H350**: May cause cancer
  - **A26**: For professional users only
  - **P201**: Obtain special instructions before use
  - **P280**: Wear protective gloves/protective clothing/eye protection/face protection
  - **P308+P313**: IF exposed or concerned: get medical advice/attention
  - **P305+P351+P338**: IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
  - **P337+P313**: If eye irritation persists: get medical advice/attention
  - **P302+P352**: IF ON SKIN: wash with plenty of soap and water
  - **P333+P313**: If skin irritation or rash occurs: get medical advice/attention
- Please follow your local safety recommendations when you handle paraformaldehyde 2 % reagent.
- Follow the appropriate regulation for waste disposal.

6 PROCEDURE

Note: one calibration curve must be performed per sample series.

6.1 Choice of the antibodies

To be used with the kit, the specific MAbs must be used at **saturating concentration in the matrix to be used**.

6.2 Protocol

Note: For good results exercise great care in the pipetting of small reagent volumes by depositing them at the bottom of the tubes.

A/ Setup of antibody and calibrator tubes

• Setup of antibody tubes : **MAb** tube
In a rack, prepare the number of 15 mL plastic tubes needed.

For each sample, pipette **100 µL** of **whole blood** or **isolated cells**.

• Setup of calibration tube : **Calibrator** tube
One calibration curve is necessary per sample series.
In a separate 15 mL tube: pipette **50 µL** of **Reagent 2 after resuspension using a vortex for 5 seconds**.

B/ Immunostaining

For each MAb tube,

- Pipette **20 µL** of the purified **MAb at 6 fold concentrated saturation**.
- Homogenize the tubes using a vortex for 1 to 2 seconds.
- Incubate all tubes according to your needs (time and temperature).

C/ Red blood cell lysis (if necessary)

If the sample is isolated cells, perform directly step D.

- Distribute **4 mL** of diluted **Reagent 5** and vortex at max speed.
- Incubate **5 minutes** (temperature according to your needs).
- Vortex again at max speed and incubate for another **5 minutes**.
- Vortex at max speed.
- Centrifuge the tubes for **5 minutes** at **700 g** and remove the supernatant.
- Keep tubes upside down on a paper towel for about 2 minutes in order to remove the maximum of liquid.

D/ Washing

- To each **MAb** tubes: add **4 mL** of diluted **Reagent 1**.
- Homogenize the tubes using a vortex for 1 to 2 seconds.
- Centrifuge the tubes for **5 minutes** at **700 g** and remove the supernatant.
- Keep tubes upside down on a paper towel for about 2 minutes in order to remove the maximum of liquid.
- Measure the residual liquid volume, the highest acceptable volume is 30 µL maximum.
- Adjust the volume with the right amount of **Reagent 1** to obtain 100 µL final volume.
- Resuspend the cells pellet using a vortex for 1 to 2 seconds.

E/ FITC staining

- For each **MAb** tube, distribute **20 µL** of **Reagent 3**.
- For the **calibrator** tube, distribute **10 µL** of **Reagent 3**.
- Homogenize the tubes using a vortex for 1 to 2 seconds.
- Incubate the tubes for **10 minutes** (temperature according to your needs).

F/ Washing

- To each tube : add **4 mL** of diluted **Reagent 1**.
- Homogenize the tubes using a vortex for 1 to 2 seconds.
- Centrifuge the tubes for **5 minutes** at **700 g** and remove the supernatant.
- Keep tubes upside down on a paper towel for about 2 minutes in order to remove the maximum of liquid.

For a single color protocol, perform directly step I.

G/ Neutralization and counter-staining (if necessary)

Neutralization:

To each **MAb** tubes,

- Add **50 µL** of **Reagent 4**.
- Homogenize the tubes using a vortex for 1 to 2 seconds to resuspend the cell pellet.

Counter-staining:

To each neutralized **MAb** tubes,

- Add the counter-staining reagent (not provided) according to the manufacturer's recommendations.
- Homogenize the tubes using a vortex for 1 to 2 seconds.
- Incubate the tubes according to the manufacturer's recommendation.

H/ Washing

To each counter-stained tubes,

- Add **4 mL** of diluted **Reagent 1**.
- Homogenize the tubes using a vortex for 1 to 2 seconds.
- Centrifuge the tubes for **5 minutes** at **700 g** and remove the supernatant.
- Keep tubes upside down on a paper towel for about 2 minutes in order to remove the maximum of liquid.

I/ Fixation

To each tubes (**MAb** and **calibrator**),

- Add **500 µL** of **Reagent 6**.
- Homogenize the tubes using a vortex for 1 to 2 seconds.
- Incubate the tubes for **30** minutes at 5 ± 3°C.

J/ Final dilution

To each tubes (**MAb** and **calibrator**),

- Add **4 mL** of diluted **Reagent 1**.
- Homogenize the tubes using a vortex for 1 to 2 seconds.

K/ Storage before cytometric analysis

The tubes can be stored **24 hours at 5 ± 3°C** before cytometric analysis. For longer period, validate the impact of the storage on the results.

L/ Cytometric analysis

Before the cytometric analysis,

- Centrifuge the tubes for **5 minutes** at **700 g** and remove the supernatant.
- Keep tubes upside down on a paper towel for about 2 minutes in order to remove the maximum of liquid.
- Add **300 to 500 µL** of diluted **Reagent 1**.
- Resuspend the cell pellets using a vortex for 1 to 2 seconds.

Refer to the Operator's Manual of the cytometer for instructions on how to perform cytometric readings.

The selected Mean Fluorescence Intensity (MFI) statistics is the geometric mean (Mn (x) or GeoMean depending upon the cytometer).

7 RESULTS

Computer data analysis or graphic data analysis

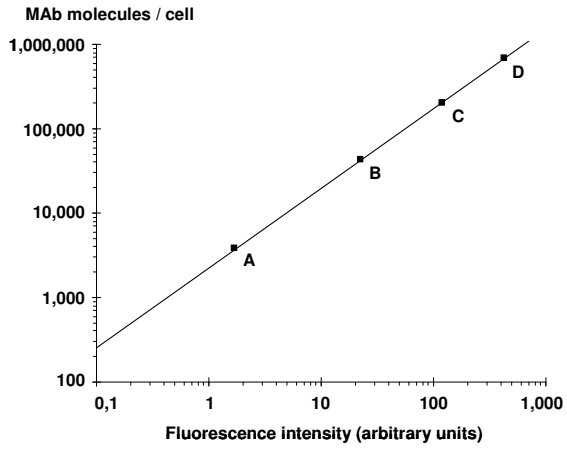
Plot the LOG_{10} of the MFI calibration values for the 4 calibration beads on the x-axis, and their corresponding LOG_{10} number of MAb molecules as indicated on the calibration flyer, on the y-axis.

Draw the optimal calibration curve of the type $LOG_{10}(ABC) = a \times LOG_{10}(MFI) + b$.

Interpolate the LOG_{10} of the MFI values of the sample tubes on the calibration curve and read directly their corresponding numbers of bound MAb molecules (ABC: Antibody Binding Capacity).

The specific quantitative values (sABC: specific Antibody Binding Capacity) are determined by subtracting the negative isotypic control ABC value.

Example of calibration curve:



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