HUMAN IgG Calibrator

Calibration kit for the quantitation of Human IgG on whole blood or on cell lines by flow cytometry

For 10 calibration curves and 50 tests CP010

BioCytex

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

1 INTENDED USE

Calibration kit for the measurement of human IgG expression level by multiple color analysis.
Whole blood is stained by indirect immunofluorescence 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 IgG is determined using the calibration beads.
HUMAN IgG Calibrator is also applicable on isolated cells.

2 REAGENTS

- **Reagent 1:** 2 x 55 mL vial, diluent, 10 fold concentrated.
- **Reagent 2:** 1 x 0.5 mL vial, calibration beads. The beads are coated with increasing and accurately known quantities of Human IgG. The number of determinants coated on each bead population is indicated in the calibration flyer provided in the kit. These values may slightly vary from lot to lot.
- **Reagent 3:** 1 x 1.1 mL vial, staining reagent, polyclonal antibody anti human IgG-FITC.
- **Reagent 4:** 1 x 2.5 mL vial, neutralization solution.
- **Reagent 5 :** 2 x 10 mL vial, lysing solution, 10 fold concentrated.
- **Reagent 6 :** 1 x 30 mL vial, fixative solution.

HUMAN IgG Calibrator contains enough reagents to perform:
- 10 calibration curves.
- 50 stainings.

REAGENTS REQUIRED BUT NOT PROVIDED

- Non conjugated human monoclonal antibodies.
- Non conjugated human negative isotypic control.
- Counter-staining reagent from mouse origin (other than FITC).
- Anti human IgG-FITC reagent (to determine the saturating concentration).
- Lysing solution (to determine the saturating concentration).
- Washing buffer (to determine the saturating concentration).

WARNING

- Reagent 6 contains 1% paraformaldehyde. The following regulation may apply:

H351: Suspected of causing cancer
H319: Causes serious eye irritation
H317: May cause an allergic skin reaction
H333: May be harmful if inhaled
P201: Obtain special instructions before use
P280: Wear protective gloves/protective clothing/eye protection/face protection
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P302 + P352: IF ON SKIN: Wash with plenty of soap and water

- Please follow your local safety recommendations when you handle paraformaldehyde 1 % reagent.
- Follow the appropriate reglementation for waste disposal.

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. Do not freeze the kit.

- **Reagents 1 and 5:** Stability after opening: 2 months at 2-8 °C when free of contamination.
  Prepare a **1:10 dilution** with distilled water.
  Prepare the appropriate volume required for the series to be tested.
  Stability after dilution: 2 months at 2-8 °C when free of contamination.
  The presence of crystals in Reagent 1 does not affect the quality of the reagent. Incubate at 37 °C until the crystals are completely dissolved.
- **Reagent 2: <u>After resuspension by vortex for 5 seconds</u>,** the reagent is ready for use.
  Stability after opening: 2 months at 2-8 °C when free of contamination.
- **Reagents 3, 4 and 6:** Ready for use.
  Stability after opening: 2 months at 2-8 °C when free of contamination.

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 EDTA or heparin.
For samples containing more than $5x10^6$ cells/mL, the sample numeration must be adjusted with diluted Reagent 1.
- Alternatively, the test can be performed on isolated cells.
The cell suspension must be adjusted at $3x10^6$ 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.
- Isolated cells (or cultured cells) must be stored according to their particular characteristics.

5 PROCEDURE

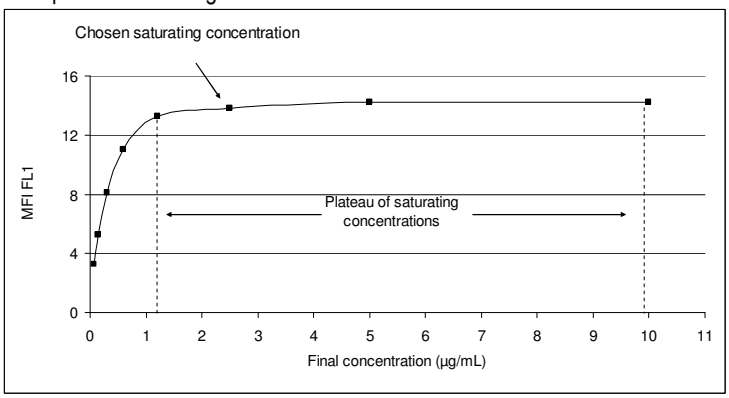
Note : one calibration curve must be performed per sample series.

5.1 Choice of the antibodies

<u>5.1.1 Choice of the specific antibody</u>

- To be used with the kit, the specific antibody must be used at **saturating concentration**.
The determination of the saturating concentration must be performed by indirect immunofluorescence with washings:
- 2 by 2 dilutions of the specific antibody.
- incubation of the dilutions with the sample for a specific time and temperature to apply later on the kit protocol.
- red blood cell lysis.
- washing.
- staining with an anti human IgG-FITC reagent of your choice.
- washing and cytometric analysis.
On the curve relating the MFI of the stained cells to the final antibody concentration, choose the minimal saturating concentration situated at the plateau of the curve.
- The determined saturating concentration multiplied by 6 will be the initial antibody concentration to be used in the kit protocol.

Example of a saturating curve:



<u>5.1.2 Choice of the negative isotypic control</u>

- The negative isotypic control to use must have the same isotype than the specific antibody and must be adjusted at the same concentration.
- The negative isotypic control must be performed for each blood sample.
- If you do not have access to a negative isotypic control, use diluted Reagent 1 instead.

5.2 Protocol

Note: For good results exercise great care in the pipetting of small reagent volumes by depositing them at the bottom of the test tubes.
All reagents must be at room temperature.

A/ Sample preparation

Prepare 2 x 15 mL tubes labeled T1 and T2.

In tube T1:
- pipette **100 µL** of whole blood or isolated cells.
- add **20 µL** of the humanized IgG Mab.

In tube T2:
- pipette **100 µL** of whole blood or isolated cells.
- add **20 µL** of the negative isotypic control Mab (or diluted Reagent 1).
- homogenize T1 and T2 tubes using a vortex for 1 to 2 seconds.
- incubate for the appropriate time and temperature determined at § 5.1.1.

B/ Sample lysis

For whole blood:
In T1 and T2 tubes:
- pipette **4 mL** of diluted Reagent 5.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- incubate the tubes on ice for **5 minutes**.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- incubate the tubes on ice for **5 minutes**.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- centrifuge the tubes at **4°C** for **5 minutes** at **300 g** and discard the supernatant (turn the tube upside down and remove the remaining last drop on tissue paper).
- add **4 ml** of diluted Reagent 1.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- centrifuge the tubes at **4°C** for **5 minutes** at **300 g** and discard the supernatant (turn the tube upside down and remove the remaining last drop on tissue paper).

For isolated cells:
In T1 and T2 tubes:
- pipette **4 mL** of diluted Reagent 1.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- centrifuge the tubes at **4°C** for **5 minutes** at **300 g** and discard the supernatant (turn the tube upside down and remove the remaining last drop on tissue paper)
- add **4 ml** of diluted Reagent 1.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- centrifuge the tubes at **4°C** for **5 minutes** at **300 g** and discard the supernatant (turn the tube upside down and remove the remaining last drop on tissue paper).

In case of non specific binding from the humanized IgG Mab, proceed twice to the following steps in tubes T1 and T2:
- add **3 ml** of diluted Reagent 1.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- centrifuge the tubes at **4°C** for **5 minutes** at **300 g** and discard the supernatant (turn the tube upside down and remove the remaining last drop on tissue paper).

For whole blood or isolated cells:
In T1 and T2 tubes:
-resuspend the cell pellet with pipette by adding **100 µL** of diluted Reagent 1.
- homogenize the tubes using a vortex for 1 to 2 seconds.

C/ Fluorescent staining

Calibration tube
In a 4-mL tube labeled T3:
- pipette **50 µL** of Reagent 2 **after resuspension using a vortex for 5 seconds**
- add **10 µL** of Reagent 3.
- homogenize the tube using a vortex for 1 to 2 seconds.

In the meantime for the sample
In T1 and T2 tubes:
- add **20 µL** of Reagent 3.
- homogenize the tubes using a vortex for 1 to 2 seconds.

For T1, T2 and T3 tubes:
- incubate the tubes on ice for **10 minutes**.
- add **3 mL** of diluted Reagent 1.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- centrifuge the tubes at **4°C** for **5 minutes** at **300 g** and discard the supernatant (turn the tube upside down and remove the remaining last drop on tissue paper).

Store tube T3 (Calibration) on ice until step E.

For a single color protocol, perform directly step E.

D/ Neutralization- counter-staining and washing (if necessary)

In T1 and T2 tubes:
- resuspend the cell pellet with pipette by adding **50 µL** of Reagent 4.
- homogenize the tube using a vortex for 1 to 2 seconds.
- pipette a volume of counter-staining reagent(s), according to the manufacturer's recommendation.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- incubate the tubes according to the manufacturer's recommendation.
- add **3 mL** of diluted Reagent 1.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- centrifuge at **4°C** the tubes for **5 minutes** at **300g** and discard the supernatant (turn the tube upside down and remove the remaining last drop on tissue paper).

E/ Fixation and flow cytometric analysis

In T1, T2 and T3 tubes:
- homogenize the tubes using a vortex for 1 to 2 seconds.
- add **500 µL** of Reagent 6.
- homogenize the tubes using a vortex for 1 to 2 seconds.
- incubate either **1 hour at room temperature** or **overnight at 2-8°C.**
- perform flow cytometric analysis.

Depending upon the experimental conditions, the lysis step could also be performed either at the beginning or at the end of the protocol.
Please contact us if you require further information.

6 CYTOMETRIC ANALYSIS

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).

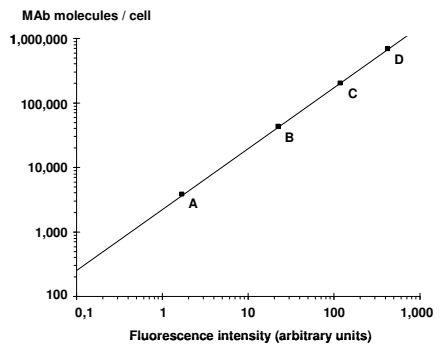
7 RESULTS

Computer data analysis or graphic data analysis.

7.1. Computer data analysis:
The result treatment is easily performed using a calculation template available upon request from the BioCytex technical department.

7.2 Graphic data analysis:
If the MFI values are expressed as linearized values or channel numbers, use a log-log or semi-log graph paper, alternatively.
Plot the MFI calibration values (calibration tube) on the abscissa (x-axis) and their corresponding number of monoclonal antibody molecules (as indicated in calibration flyer) on the ordinate (y-axis).
Draw the calibration curve.
Interpolate the MFI values of the test tubes on the calibration curve and read off directly their corresponding molecule numbers.
Specific quantitative values (sABC) of the binding of the selected antibodies are calculated after subtraction of the corresponding negative isotypic control measurement.

Example of calibration curve:



BIOCYTEX
140 ch. ARMEE D'AFRIQUE
13010 MARSEILLE
FRANCE
TEL : +33 (0) 4 96 12 20 40
FAX : +33 (0) 4 91 47 24 71

CPB0158
Version May 2025