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PeliDuo Control

Mixture of conjugated monoclonal mouse IgG antibody reagent to be used as negative control.

REF M1627 IgG1F / IgG1PE

Form Clone
IgG1 FITC CLB-203
IgG1 PE CLB-203

REF M2251 IgG2aF / IgG1PE

Form Clone
IgG2a FITC CLB-713
IgG1 PE CLB-5A497

X0033-524eng 0110031639



1. INTENDED USE

The PeliDuo antibody mixtures are intended for in vitro diagnostic use as negative control reagent for flow cytometry.

To prevent interference with red cells during analysis treatment of whole blood with PeliLyse reagent (order number M7101.6) is recommended.

The flow cytometer must be equipped to detect light scatter and the appropriate fluorescence, and be equipped with the appropriate analysis software for data acquisition and analysis. Refer to your instrument user's guide for instructions.

Applications

Assessment of non-specific binding of mouse monoclonal antibodies to human blood cell surface antigens in flow cytometry.

2. COMPOSITION

Clone CLB-203 (M1627) has been derived from ascites fluid of tumour bearing mice and is of a mouse IgG1 subclass. The antibody is conjugated with fluorescein iso-thiocyanate isomer 1 (FITC). The molecular F/P ratio is between 5 and 10. The antibody is separately conjugated with R-phycoerythrin (PE). The molecular F/P ratio is between 1.0 – 2.0.

Clone 713 (M2251) has been derived from ascites fluid of tumour bearing mice and is of a mouse IgG2a subclass. The antibody is conjugated with fluorescein iso-thiocyanate isomer 1 (FITC). The molecular F/P ratio is between 5 and 10.

Clone CLB-5A497 (M2251) has been derived from ascites fluid of tumour bearing mice and is of a mouse IgG1 subclass. The antibody is conjugated with R-phycoerythrin (PE). The molecular F/P ratio is between 1.0 – 2.0.

The antibodies were purified using column chromatography (ion exchange and affinity chromatography).

Reagent contents.

Both reagents are supplied as an optimally formulated mixture of both conjugated antibodies in 1 ml of 20 mM TRIS and 150 mM NaCl buffer, pH 8.0, containing stabilising protein (0.4%) and Na₂S₂O₃ 0.1% (w/v) as preservative. The concentration and F/P ratio of our controls have been adjusted to our conjugated monoclonal antibodies.

Table 1. Contents of bottles

| | |
|---------|--------------------------------|
| PeliDuo | 50 tests per ml in TRIS buffer |
|---------|--------------------------------|

WARNING:

Sodium azide is harmful if swallowed (R22). Keep out of reach of children (S2). Keep away from food, drink, and animal feedingstuff (S13). Wear suitable protective clothing (S36). If swallowed, seek medical advice immediately and show this container or label (S46). Contact with acids liberates very toxic gas (R32). Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.

3. STORAGE AND HANDLING

The reagent is stable until the expiration date shown on the label when stored at 2 to 8°C in the dark. Do not use after the expiration date. Do not freeze the reagent or expose it to direct

light during storage or incubation with cells. Keep the outside of the reagent vial dry. Reagents should not be used if any evidence of deterioration, such as increase in compensation, or substantial loss of reactivity is observed.

4. REAGENTS OR MATERIALS REQUIRED BUT NOT PROVIDED

- Lysing solution (PeliLyse, order number M7101.6).
- Wash and dilution buffer for mononuclear cells, Phosphate Buffered Saline, containing 0.2% BSA (w/v) (PBS/BSA).
- Wash and dilution buffer for platelets, Sequesterine buffer (Seq), storage 1 month at 2 to 8°C. 10 x stock solution, dissolve in 1 litre of distilled water:
Na₂HPO₄ · H₂O : 15.65 g
Na₂EDTA · 2H₂O : 16.65 g
(Complexon)
NaCl : 450.0 g
- Prior to use dilute in distilled water, add BSA till final concentration of 0.2% (w/v). Mix and adjust pH to 6.8.
- Fixation buffer, PFA/BSA (*): Para-Formaldehyde 1% in PBS, containing 0.2% BSA (pH 7.2).
- Microwell plates (96 wells, V bottom) or plastic flow cytometry tubes.
- Flow cytometer. Refer to the appropriate instrument user's guide for information.

(* The procedure employs a fixative, formaldehyde. Contact is to be avoided with skin or mucous membranes.

5. SPECIMEN(S)

Blood samples can be prepared for flow cytometric analysis by using PBMC preparation procedures. PBMC preparation yield more technique-dependent results (1). Collect blood aseptically by venipuncture (1,2) into sterile K₂EDTA blood collection tube. A minimum of 1 ml of whole blood is required for the whole blood method and a minimum of 2 ml of whole blood is required for PBMC preparation. Store anticoagulated blood at room temperature (18 to 25°C).

WARNING:

Consider all biological specimens and materials which come in contact with them as biohazardous. Specimens should be handled as potentially infectious (3,4) and disposed with proper precaution in accordance with federal, state and local regulations. Do not pipet by mouth. Wear suitable protective clothing and gloves. Fixation has been reported to inactivate HIV (5).

6. PROCEDURES

A: Method with ficoll purified cells

- 1 Prepare a mononuclear cell suspension with a concentration of 1 x 10⁷ cells/ml.
- 2 Add 30 µl of cell suspension to microtiter wells or tubes.
- 3 Add 20 µl of the undiluted antibodies to the microtiter wells or tubes and mix gently.
- 4 Incubate for 30 minutes at 2 to 8°C.
- 5 Add 150 µl buffer to the microtiter wells or 2 ml buffer to the tubes and centrifuge at 500 x g for 5 minutes.
- 6 Aspirate the supernatant from the cell pellet and resuspend the cells.
- 7 Add 200 µl buffer to the microtiter wells or 2 ml buffer to the tubes and centrifuge at 500 x g for 5 minutes.
- 8 Aspirate the supernatant from the cell pellet and resuspend the cells.
- 9 Flow cytometer analysis:
Add 200 µl buffer to the microtiter wells and transfer this final cell suspension to appropriate testtubes, or add 200 µl buffer to the tubes.
- 10 If analysis within 8 hours is not possible add at no. 9, instead of buffer, 200 µl PFA 1%. Sanquin Reagents recommends then analysing within 24 hours.

B: Whole blood method

- 1 Draw blood into a blood collection tube containing EDTA.
- 2 Deliver 100 µl (*) of well mixed whole blood to the bottom of the test tube.
- 3 Add 20 µl of the undiluted antibodies to the bottom of the test tube, and mix firmly during 30 seconds.
- 4 Incubate for 15 to 30 minutes at room temperature.
- 5 Mix the tubes and add 2 ml of lysing solution (PeliLyse A1, 10x diluted).
- 6 Incubate for 10 to 15 minutes at room temperature until lysing is complete.
- 7 Analyse the samples within 90 minutes.

If analysis within 90 minutes is not possible, centrifuge the tubes at 500x g for 5 minutes. Aspirate the supernatant from the

cell pellet and resuspend the cells in 1 ml buffer when analysed within 8 hours or in 1 ml PFA 1%. Sanquin Reagents recommends then analysing within 24 hours.

* This method was developed for blood samples with a normal white count with the use of PeliLyse A1 (lysing solution, order number M7101.6). It may be necessary to adjust the quantity of blood for samples with very high or low white count.

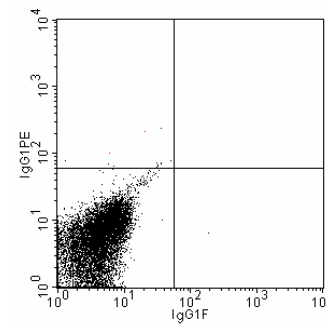
Analytical Results

Fluorescence obtained with these negative controls reflects the amount of a-specific binding of mouse antibodies to the cell sample and should be less than approx. 5% of the counted cells.

Flow cytometry

Vortex the cells thoroughly at low speed to reduce aggregation before running the cells on the flow cytometer (6). Acquire and analyse list-mode data using appropriate software. Before acquiring samples, adjust the threshold to minimise debris and ensure populations of interest are included. Fig 1 displays representative data performed on gated lymphocytes. Laser excitation is at 488 nm.

Fig. 1: Fluorescence profile, scatter gates set on the lymphocyte fraction (R1)



NOTE: Improper gate setting on the sample data can give incorrect results.

7. PERFORMANCE CHARACTERISTICS Specificity

The monoclonal antibodies with subclass IgG1, clone CLB-203 and CLB-5A497, are directed against plant allergens. There is no reactivity with human blood cells and immunoglobulins.

The monoclonal antibody, clone CLB-713, is isolated from a non-immunized mouse. There is no reactivity with human blood cells and immunoglobulins.

Reproducibility/Repeatability.

To determine the repeatability of staining with each reagent, samples were stained with multiple lots of reagents. The different samples used in the evaluation provided an average mean fluorescence intensity (MFI) value as shown in table 2. For each sample, two different lots of reagent generated a pair of results. Individual SDs were determined from the paired results for each sample. The SDs were combined to derive a pooled SD for each reagent that provides an estimate of within-sample repeatability.

Table 2. Repeatability of mean fluorescence intensity (MFI) of target cells across different lots (N) and across multiple donors.

| | N* | Average MFI | Pooled SD | Pooled %CV |
|-----------------|----|-------------|-----------|------------|
| Ctrl IgG1 FITC | 15 | 6.21 | 0.18 | 2.86% |
| Ctrl IgG2a FITC | 15 | 7.95 | 0.7 | 8.84% |
| Ctrl IgG1 PE | 15 | 9.71 | 0.43 | 4.46% |

* N = number of samples

8. LIMITATIONS

Conjugates with brighter fluorochromes (PE, PE-Cy5) will give a greater separation than those with other dyes (FITC). When populations overlap, calculation of the percentage positive for the markers can be affected by choice of fluorochrome.

Use of antibodies in patient treatment can interfere with recognition of target antigens by CD reagents. This should be considered when analysing samples from patients treated in this fashion.

Sanquin Reagents has not characterised the effect of the presence of therapeutic antibodies on the performance of this reagent.

As reagents can be used in different combinations, laboratories need to become familiar with the properties of each antibody in conjunction with other markers in normal and abnormal samples.

Reagent data performance was collected typically with EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

TROUBLESHOOTING

| Problem | Possible Cause | Solution |
|--|---|---|
| Poor resolution between debris and lymphocytes | Cell interaction with other cells and platelets Rough handling of cell preparation Inappropriate instrument settings | Prepare and stain another sample. Check cell viability; centrifuge cells at lower speed. Follow proper instrument set-up procedures; optimise instrument settings as required. |
| Staining dim or fading | Cell concentration too high at staining step Insufficient reagent Cells not analysed within 8 hours of staining Improper medium preparation (preservative omitted) | Check and adjust cell concentration or sample volume; stain with fresh sample Repeat staining with increased amount of antibody. Repeat staining with fresh sample; analyse promptly. Use preservative in staining medium and washing steps. |
| Few or no cells | Cell concentration too low Cytometer malfunction | Resuspend fresh sample at a higher concentration; repeat staining and analysis. Troubleshoot instrument. |

REFERENCES

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