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PRODUCT INSERT

REF

FLOWPRA® CONTROL BEADS

CATALOG # FLCNTBD

IVD **For In Vitro Diagnostic Use.**

INTENDED USE

FlowPRA® Control Beads are intended for use as blank control beads in the FlowPRA® Class I and/or II Screening Tests to indicate the background level of the testing serum.

SUMMARY AND EXPLANATION

FlowPRA® Control Beads are made of the same kind of microbeads (2-4 µm) as the beads used in the FlowPRA® Screening Test, except that there are no HLA antigens coated on the bead.

PRINCIPLE

The FlowPRA® Screening Tests are designed for flow cytometric detection of panel reactive antibody (PRA) using FlowPRA® beads (1,2). FlowPRA® Beads are microbeads coated with purified HLA Class I or Class II antigens. HLA IgG antibodies in serum can be detected by incubation of the serum with the FlowPRA® Beads, followed by staining with a fluorescent labeled anti-human IgG antibody. The anti-HLA IgG positive serum shows a fluorescent channel shift as compared to the negative serum.

Different negative sera may show slightly different background fluorescent levels in the FlowPRA® tests. FlowPRA® Control Beads are designed to monitor the background level of the negative serum due to the non-specific interaction of the serum with the beads.

FlowPRA® Control Beads are fluorescent particles, which can be excited at 488 nm, generating a maximum emission of approximately 580 nm. The fluorescent emission spectrum is unique for FlowPRA® Control Beads, allowing the beads to be distinguished from the FlowPRA® Screening Beads. Therefore, FlowPRA® Control Beads can be mixed with FlowPRA® Screening Beads to be incubated with a test serum.

REAGENTS

A. Identification

FlowPRA® Control Beads (50 tests)

B. Warning or Caution

1. For In Vitro Diagnostic Use.
2. Refer to the Material Safety Data Sheet for detailed information.

C. Instructions for Use

See Directions for Use.

D. Storage Instructions

Store reagents at temperature indicated on package. Use before printed expiration date.

Note: *The volumes provided are slightly more than the amount required for testing. This is to account for inadvertent loss which may result from pipetting.*

E. Purification or Treatment Required for Use

Testing serum may be fresh or thawed. However, aggregates should be removed from the testing serum by centrifugation or filtration prior to testing. Any aggregates in the serum or contamination of the serum may generate invalid results.

F. Instability Indications
(Physical/biological/chemical indications of instability/deterioration)

INSTRUMENT REQUIREMENTS

- Flow cytometer
- Microcentrifuge

SPECIMEN COLLECTION AND PREPARATION

Testing serum may be fresh or thawed. However, aggregates should be removed from the testing serum by centrifugation or filtration prior to testing. Any aggregates in the serum or contamination of the serum may generate invalid results.

PROCEDURE

A. Materials Provided
FlowPRA® Control Beads

B. Materials Required, But Not Provided

1. FlowPRA® Screening Test (One Lambda, Inc. Cat #: FL1-30, FL2-30, or FL12-60)
2. Class I or Class II Positive, or Negative Control Serum (One Lambda, Inc. Cat #: FL1-PC, FL2-PC, or FL-NC.)
3. Fixing solution: PBS with 0.5% formaldehyde (add 1.35 ml 37% formaldehyde to 100 ml PBS)

C. Step-by-step procedure.
See "Directions For Use" below.

DIRECTIONS FOR USE

A. Vortex FlowPRA® Control Beads prior to use.
To use with FlowPRA® screening beads: for each test, mix 1 µl of FlowPRA® Control Beads with 5 µl of FlowPRA® Class I or Class II beads[2]. For simultaneous Class I and II antibody screening, combine 5µl each of Class I and Class II beads with 1 µl of FlowPRA® Control Beads.

B. Follow the FlowPRA® Screening testing procedure.

DAILY ALIGNMENT AND QUALITY CONTROL

Follow the FlowPRA® Screening testing procedure.

RESULTS

A. Data Acquisition

1. Adjust the fluorescence compensation by using either commercially available compensation beads or using the FlowPRA® Beads according to FlowPRA® Screening Test procedure.

2. Measure green and yellow fluorescence for 5,000-10,000 events for each sample.

B. DATA ANALYSIS
For FlowPRA® Screening Test

1. Gate the major population of FlowPRA® Beads on the FSC vs. SSC dot plot (R1, Figure 1), and obtain an FL2 vs. FSC dot plot (Figure 2).
2. Gate FlowPRA® Class I (R2), Class II (R3) and Control Beads (R4) major population on the FL2 vs. FSC dot plot (Figure 2), and obtain FL1 histogram on each region (Figure 3).
3. Follow the FlowPRA® Screening Test data analysis procedure.

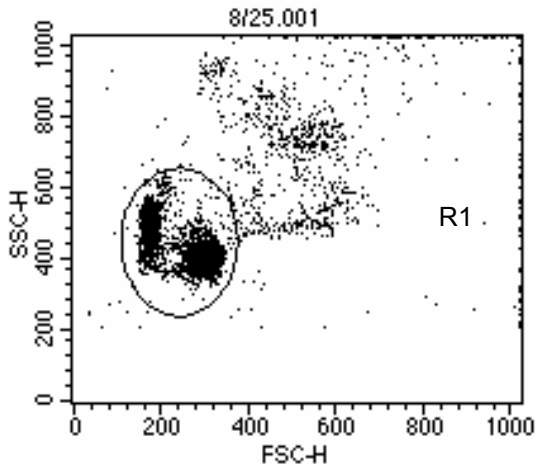

Figure 1. FSC vs. SSC Dot Plot of FlowPRA® Screening Beads

LIMITATIONS OF THE PROCEDURE

FlowPRA® Control Beads are used only as an indicator of the non-specific interaction of the testing serum with the microbeads. It should not be used as a positive/negative cut-off line for the test.

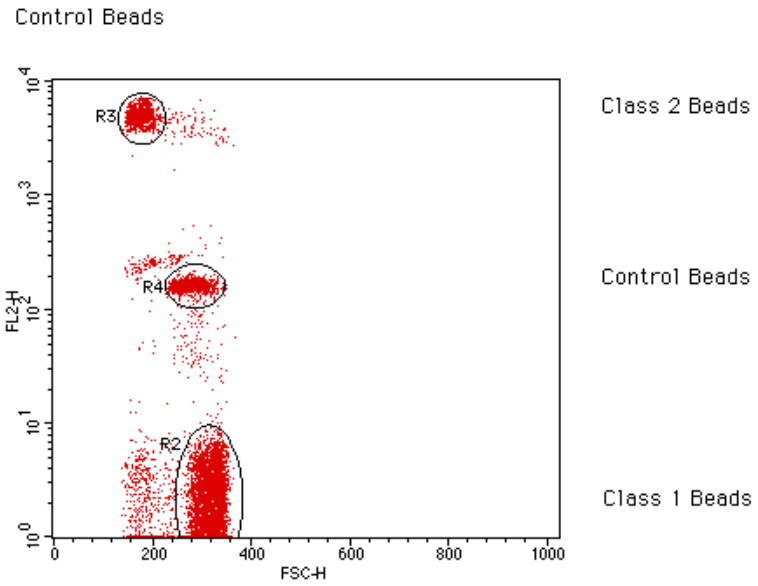

Figure 2. FL2 vs. FSC Dot Plot on FlowPRA® Screening Beads and Control Beads (Example)

EXPECTED VALUES

Most sera should not react to the control beads. However, certain patient sera may react to the control beads. In such cases, the data may not be valid. The flow histogram should be further evaluated by the laboratory director. Adsorb Out™ (OLI Cat.# ADSORB) may be used to eliminate or reduce the non-specific binding.

SPECIFIC PERFORMANCE CHARACTERISTICS

Control beads should show a negative reaction to normal human serum. Certain patients with strong antibodies against latex may show a positive reaction to the beads. In addition, insufficient washes during the testing process may also cause a slightly positive reaction.

BIBLIOGRAPHY

1. Pei R, Wang G, Tarsitani C, Rojo S, Chen T, Takemura S, Liu A, and Lee J-H. Simultaneous HLA Class I and II Antibodies Screening with Flow Cytometry. *Human Immunology* 59:313-322, 1998.
2. Pei R et al. Flow Cytometry Detection of HLA Antibodies Using a Spectrum of Microbeads. *Human Immunology*, In press.

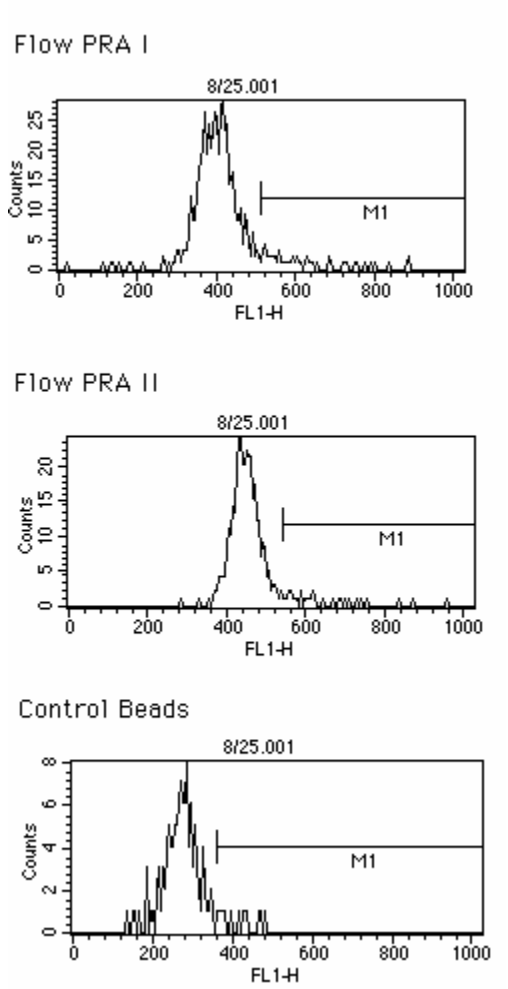

Figure 3. FL1 Histograms of FlowPRA® Class I or II Beads or FlowPRA® Control Beads Reacting with Negative Serum Control.

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REVISION HISTORY

Revision	Date	Revision Description
3	*2004/11*	*Revision to "Expected Values" Section. Update ® symbol.*
4	*2005/04*	*NC Beads are no longer part of the FlowPRA® Specific products. Remove references to FlowPRA® Specific assays. Update figures.*