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WORKING PROTOCOL

FITC-Conjugated Anti-Human C4d Antibody (C4dpAb2-FITC)
Cat.no.: BI-RC4D-FITC

[C4d]FlowPRA® test using FITC-conjugated anti-human C4d antibody (C4dpAb2)

The FlowPRA® Screening test (One Lambda, Inc., Canoga Park, CA, USA) consists of a pool of 30 different microbead preparations coated with either purified HLA class I or HLA class II antigens from different cell lines covering all common HLA antigens. According to the manufacturer´s protocol this assay is applicable for detection of anti-HLA class I or II IgG alloreactivities (Ref. 1). The [C4d]FlowPRA test was developed for detection of anti-HLA alloantibody-triggered C4d deposition to FlowPRA beads by FITC-conjugated anti-human C4d antibody. As an adjunct to standard FlowPRA® screening, this test allows for selective detection of presumably more harmful classical complement-activating anti-HLA alloantibodies. The [C4d]FlowPRA test may represent a specific alternative to conventional complement-dependent cytotoxicity (CDC) PRA testing (Ref. 2).

1. Incubate negative control serum or test serum (9 µl) with 0.5 µl FlowPRA® class I and 0.5 µl FlowPRA® class II beads*.

2. Incubate for 30 minutes on ice.

3. Add 30 µl serum obtained from a nonsensitized healthy male volunteer with normal C4 levels, and normal CH50 activity.

4. Incubate for another 30 minutes on ice.

5. Wash beads twice with cold phosphate-buffered saline.

6. Add 20 µl working dilution (1:25) of FITC-conjugated C4dpAb2 to 20 µl beads suspension (final dilution 1:50).

7. Incubate for 30 min on ice.

8. Wash beads twice with cold phosphate-buffered saline and analyze by flow cytometry.

9. The major bead population is gated on the forward-versus-side-scatter dot plot. Then, two gates are set on the FL2 histogram to separately analyze HLA class I-coated (FL2-negative) and class II (FL2 high-fluorescent)-coated populations. FITCconjugated anti-human C4d antibody binding to HLA class I or class II beads is evaluated on FL1 histograms. The marker is set according to staining with the negative control serum. Results are expressed as percentage of positive events.

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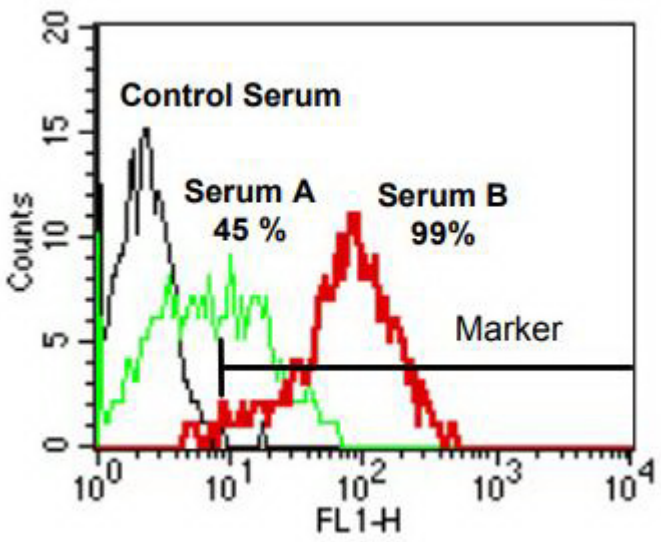

Figure. [C4d]FlowPRA (HLA class I) screening of representative serum (serum A, serum B) obtained from two presensitized patients. A FL-1 histogram (FlowPRA® HLA class I beads are gated) is depicted. The marker is set according to C4d staining obtained with a non-binding control serum. The percentage of positive events, which represents %[C4d]FlowPRA reactivity is indicated for the two test sera.

Additional reagents required:
FlowPRA® Class I Screening Test, Cat. No. FL1-30
FlowPRA® Class II Screening Test, Cat. No. FL2-30

One Lambda, Inc., 21001 Kittridge Street, Canoga Park, CA, 91303-2801 USA, www.onelambda.com

Literature

1. Simultaneous HLA Class I and Class II antibodies screening with flow cytometry. Pei R et al., Hum Immunol. 1998 May;59(5):313-22.
2. [C4d]FlowPRA screening – a specific assay for selective detection of complement-activating anti HLA alloantibodies. Wahrmann M. et al., Hum Immunol, 2005 May;66(5):526-534

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