Matched-Pair Antibody Set
for ELISA of human Fibrinogen antigen (Fg)

Sufficient reagent for 5 x 96 well plates

**Supplied Materials:**

1. **Capture Antibody (FG-EIA-C):** One yellow-capped vial containing 0.5 ml of affinity-purified polyclonal anti-fibrinogen antibody for coating plates.

2. **Detecting Antibody (FG-EIA-D):** One red-capped vial containing 0.5 ml of affinity-purified peroxidase conjugated polyclonal anti-fibrinogen antibody for detection of captured fibrinogen.

**Note:** Antibodies are supplied in a 50% (v/v) glycerol solution for storage at -10 to -20°C. Keep vials tightly capped. Do not store in frost-free freezers.

**Materials Required but not Provided:**

This paired antibody set has been optimized for performance using the buffers and conditions described below. Most reagents are available as part of the VisuLize™ Buffer Pak (see Related Products section) or may be prepared as described below.

1. **Coating Buffer:** 50 mM Carbonate 1.59g of Na2CO3 and 2.93g of NaHCO3 up to 1 litre. Adjust pH to 9.6. Store at 2-8°C up to 1 month.

2. **PBS:** (base for wash buffer and blocking buffer) 8.0g NaCl, 1.15g Na2HPO4 0.2g KH2PO4 and 0.2g KCl, up to 1 litre. Adjust pH to 7.4, if necessary. Store up to 1 month at 2-8°C, discard if there is evidence of microbial growth.

3. **Wash Buffer:** PBS-Tween (0.1%,v/v)
   To 1 litre of PBS add 1.0 ml of Tween-20. Check that the pH is 7.4. Store at 2-8°C up to 1 week.

4. **Blocking Buffer:** HBS-BSA-T20 5.95g HEPES (free acid), 1.46 g Na Cl, 2.5 g Bovine Serum Albumin (Sigma-RRIA grade) in 200 ml of PBS. Adjust pH to 7.4, if required, then make up to 250 ml with PBS. Aliquot and store frozen at -20°C.

5. **Sample Diluent:** HBS-BSA-T20 6.95g HEPES (free acid), 1.46 g Na Cl, 2.5 g Bovine Serum Albumin (Sigma, RIA grade) dissolved in 200 ml H2O. Add 0.25 ml of Tween-20, check and adjust pH to 7.2 with NaOH, then make up to a final volume of 250 ml with H2O. Aliquot and store frozen at -20°C.

6. **Substrate Buffer:** Citrate-Phosphate buffer pH 5.0 2.6g Citric acid and 6.9g Na2HPO4 up to a final volume of 500 ml with purified H2O. Store at 2-8°C up to 1 month.

7. **OPD Substrate:** (o-Phenylenediamine,2HCl) Toxic! (5mg tablets: Sigma # P-6912). Make up immediately before use. Dissolve 5mg OPD in 12 ml substrate buffer then add 12 µl 30% H2O2. Do not store.

8. **Stopping Solution:** 2.5 M H2SO4
   Caution: very corrosive! Generates heat on dilution! Where stock sulphuric acid is 18 Molar, add 13.9 ml to 86 ml H2O. Store at room temperature.

9. **Other:**
   Optional: VisuLize™ Buffer Pak (see Related Products section)
Assay Procedure:

1. Coating of plates:
   Dilute the capture antibody 1/100 in coating buffer (preferably in a polystyrene tube) and immediately add
   100 µl to every well in the plate. Incubate 2 hours at 22°C or overnight at 2-8°C.

2. Blocking:
   Empty contents of plate and add 150 µl of blocking buffer to
   every well and incubate for 60 minutes @ 22°C.
   Wash plate X 3 with wash buffer.

3. Samples:
   Reference plasma is diluted 1/10,000 (100%) then serial
   1/2's down to 1/320,000 (3.13%). Sample plasmas are
   diluted 1/20,000, 1/40,000 & 1/80,000. All dilutions are
   made in HBS-BSA-T20 sample diluent. Apply 100 µl/well and incubate plate @ 22°C for 60 minutes. Wash plate X 3 with
   wash buffer.

4. Detecting Antibody:
   Dilute the detecting antibody 1/100 in HBS-BSA-T20 sample
diluent and apply 100 µl to each well. Incubate plate @ 22°C
   for 60 minutes. Wash plate X 3 with wash buffer.

5. OPD Substrate:
   Apply 100 µl of freshly prepared OPD substrate to every well.
   Allow colour to develop for 10-15 minutes then stop colour
   reaction with the addition of 50 µl/well of 2.5 M H2SO4. The
   plate can be read at a wavelength of 490 nm.

Calculation of Results:

The construction of a proper reference curve is of no less
importance than any other aspect of the assay. A reference curve
should be constructed by plotting the known concentration of
standards versus absorbance. This can be done manually using
graph paper, or by using curve-fitting computer software. In our
experience, the dose response curves of most immunoassays tend
to be sigmoid in shape. Although linear regions can be identified
within the curve, the best overall fit is often obtained using an
algorithm that provides a weighted theoretical model of fit
throughout the entire curve, such as a 4-parameter or 5-parameter
logistic curve fit. In general, the simplest model that defines the
concentration-response relationship should be used. The
“back-fit” test is a simple and reliable method to determine if a
curve-fitting method is appropriate. In this test, the apparent
concentrations for the absorbance values of each standard point
are read from the reference curve. The derived values are
compared to the assigned values. An appropriate curve fitting
method will produce derived values that closely match assigned
values throughout the range of the curve, within user-defined
limits. The coefficient of determination (R^2) is a valuable indicator
of the overall fit, but should not be used by itself in the selection of
a curve fitting method, as a poor fit in a particular region of the
curve may not be evident from this value alone.

In the quality control of this product we have determined that
under the conditions described above, a reference curve that is
constructed using serial dilutions of normal pooled plasma, will
produce a correlation coefficient (R^2) of at least 0.980 using a log-
log fit, and an R^2 of at least 0.990 using a 4-parameter logistic
curve fit algorithm. However, the performance characteristics of
in-house assays developed using this product in other laboratories
may vary slightly from ours. Different curve fitting methods may be
employed but we recommend that the back-fit test be applied as
evidence that the fitting method is appropriate.

Technical Notes:

- This paired antibody product is intended to facilitate the end user
  in establishing an in-house immunoassay for research purposes
  only. It must not be used for diagnostic applications. Assay
  validation is the responsibility of the end user and should be
done according to user-defined protocols.
- Reference calibrators should be of the same matrix and
  anticoagulant as the samples to be tested (example serum or
  plasma, citrate or EDTA).
- Do not use samples diluted less than 1/500, as falsely high
  readings may result.
- The optimal colour development time should be determined
  empirically as the time required to obtain an absorbance of at
  least 1.000 at 490 nm for the 100% reference point, not to
  exceed 20 minutes.
- Rheumatoid factor in samples may interfere in ELISA by binding
to the capture and/or detecting antibodies.
- The wells should not be allowed to become dry. Keep plate
  covered or in a humid chamber during incubations.
- Antibodies are supplied in a 50% glycerol solution and can be
  centrifuged briefly in a micro-centrifuge to gather residual
  reagent from the cap and walls of the tube.

References:

1. Hantgan RR, Francis CW, Marder VJ; Fibrinogen Structure and Physiology;
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Marder and EW Salzman, pp 277-300, J.B. Lippincott Co., Philadelphia PA,
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2. Shafer JA, Higgins DL; Human Fibrinogen; CRC Critical Reviews in Clinical
3. Binnie CG, Lord ST; The Fibrinogen Sequences that Interact with
   Thrombin; Blood 81, pp 3186-3192, 1993.
4. Nix,B, Wild D, in Immunoassays, A Practical Approach, editor J.P. Gosling,
5. NCCLS. Evaluation of the Linearity of Quantitative Analytical Methods;
6. FDA Guidance for Industry. Bioanalytical Method Validation; May 2001,
   available on the internet: www.fda.gov/cder/guidance/index.htm

Related Products:

Cat #: EIA-PAK-1 VisuLize™ Buffer Pak: 5 plates, buffers, substrate
Cat #: EIA-CSA-1 VisuCal™ Antigen Calibrator Plasma, 1 x 1ml
Cat #: EIA-CSA-5 VisuCal™ Antigen Calibrator Plasma, 5 x 1ml
Cat #: SAFG-IG Sheep anti-human Fibrinogen, whole IgG from antiserum
Cat #: SAFG-AP Sheep anti-human Fibrinogen, affinity-purified IgG
Cat #: SAFG-HRP Sheep anti-human Fibrinogen, peroxidase-labelled IgG
Cat #: SARFG-IG Sheep anti-rabbit Fibrinogen, whole IgG from antiserum
Cat #: SARFG-AP Sheep anti-rabbit Fibrinogen, affinity purified IgG
Cat #: SARFG-HRP Sheep anti-rabbit Fibrinogen, peroxidase-labelled IgG
Cat #: RBFG-EIA Paired antibody set for ELISA of Rabbit Fg, 4 x 96 wells
Cat #: SAFNE-IG Sheep anti-Fibrin Fragment E, whole IgG from antiserum
Cat #: SAFNE-HRP Sheep anti-Fibrin Fragment E, peroxidase labelled IgG
Cat #: SAFPA-IG Sheep anti-Fibrinopeptide A, whole IgG from antiserum
Cat #: SAFPA-AP Sheep anti-Fibrinopeptide A, affinity purified IgG
Cat #: SAFPA-HRP Sheep anti-Fibrinopeptide A, peroxidase labelled IgG

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