

CTS

4 – ELISA Kit

Summary

1. REAGENT PREPARATION SUMMARY

A. Wash Buffer

Pour the entire content (50 ml) of the Wash Buffer Concentrate into a clean 1000 ml graduated cylinder. Bring final volume to 1000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. **The pH of the final solution should adjust to 7.4.**

B. Anti-sCD30-Biotin Conjugate

- Mix Biotin Conjugate well by pipetting.
- Transfer **6 µl** directly into the vial of the supplied Assay Buffer.

C. Streptavidin-Alkaline-Phosphatase Conjugate

- Mix Streptavidin-Alkaline-Phosphatase Conjugate well by pipetting.
- Transfer **8 µl** directly into the vial of the supplied Assay Buffer.

D. Anti-human-IgG-Alkaline-Phosphatase Conjugate for detection of IgG-anti-HLA Class I antibodies

- Mix Anti-human-IgG-Alkaline-Phosphatase Conjugate well by pipetting.
- Transfer **5 µl** directly into the vial of the supplied Assay Buffer.

E. Anti-human-IgG-Alkaline-Phosphatase Conjugate for detection of IgG-anti-HLA Class II antibodies

- Mix Anti-human-IgG-Alkaline-Phosphatase Conjugate well by pipetting.
- Transfer **5 µl** directly into the vial of the supplied Assay Buffer.

F. Anti-human-IgA-Alkaline-Phosphatase Conjugate for detection of IgA-anti-Fab antibodies

- Mix Anti-human-IgA-Alkaline-Phosphatase Conjugate well by pipetting.
- Transfer **4 µl** directly into the vial of the supplied Assay Buffer.

G. Substrate Solution

- Open the pNPP tablet package (silver foil) and Tris Buffer tablet package (gold foil) and drop the tablets into an appropriate **light protected container** containing 20 ml distilled or deionized water.
- **Do not touch the tablets.**
- Vortex until tablets are completely dissolved.

It is recommended to spin vials of Conjugates and Assay Buffers in a microcentrifuge before use.

2. TEST PROTOCOL SUMMARY

- A. Prepare Wash Buffer (according to reagent preparation summary section A).
- B. Remove Sample Diluents, Conjugates, Assay Buffers, and Positive and Negative Controls from the freezer and incubate at room temperature for approximately 60 minutes.
- C. Subdivide a 96-well Dilution Plate (not delivered) according to Figure 1 of the Manual.
- D. Dilute Samples according to Table 1 of the Manual.
- E. Prepare anti-sCD30-Biotin Conjugate (according to reagent preparation summary section B).
- F. Pipette 300 μ l of Wash Buffer into each well of the coated Microwell Strips and incubate for 10 minutes. **Take care not to scratch the surface of the microwells.**
- G. After 10 minutes empty the wells by decanting and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. **Use the microwell strips immediately after washing. Do not allow wells to dry.**
- H. Transfer 50 μ l of the reaction mixture from the Dilution Plate to the coated Microwell Strips in the same scheme as prepared on the Dilution Plate.
- I. Pipette 50 μ l of each Positive and Negative Control into the coated Microwell Strips according to Figure 2 of the Manual.
- J. Add 50 μ l of prepared anti-sCD30-Biotin Conjugate (Step E) to the microwell strips coated with monoclonal antibody to human sCD30 (A1 to H3), **excluding the Blank well H1.**
- K. Cover with a Plate Cover and incubate at room temperature for 2 hours, if available on a rotator set at 100 rpm.
- L. Prepare Alkaline-Phosphatase Conjugates a few minutes prior to use (according to reagent preparation summary sections C-F).
- M. Remove plate cover and empty the wells by decanting. Wash microwell strips 4 times. **Use the microwell strips immediately after washing.**
- N. Add 100 μ l of diluted Streptavidin-Alkaline-Phosphatase Conjugate to the microwell strips coated with monoclonal antibody to human sCD30 (A1 to H3), **excluding the Blank well H1.**
Add 50 μ l of diluted Anti-human-IgG-Alkaline-Phosphatase Conjugate for detection of IgG-anti-HLA Class I antibodies to the microwell strips coated with human HLA Class I antigens (A4 to H6), **excluding the Blank well H4.**

Add 50 µl of diluted Anti-human-IgG-Alkaline-Phosphatase Conjugate for detection of IgG-anti-HLA Class II antibodies to the microwell strips coated with human HLA Class II antigens (A7 to H9), **excluding the Blank well H7.**

Add 50 µl of diluted Anti-human-IgA-Alkaline-Phosphatase Conjugate for detection of IgA-anti-Fab antibodies to the microwell strips coated with human Fab fragments (A10 to H12), **excluding the Blank well H10.**

- O.** Cover with a Plate Cover and incubate at room temperature for 1 hour, if available on a rotator set at 100 rpm.
- P.** Immediately after pipetting the AP-Conjugates, prepare Substrate Solution (according to reagent preparation summary section G).
- Q.** Remove plate cover and empty the wells by decanting. Wash microwell strips 4 times. **Use the microwell strips immediately after washing.**
- R.** Pipette 200 µl of prepared Substrate Solution to all wells, **including the Blank wells.** Incubate the microwell strips in the dark at room temperature for about 10 minutes.
- S.** After 10 minutes of incubation, read Optical Density [OD] at regular intervals (every 5 minutes) until the Positive (and Negative) Controls have reached the **values given in the lot-specific Manual delivered with the product.**
- T.** Read Optical Density on an ELISA reader using 405 nm as the primary wave length and 492 nm as the reference wave length. Blank the plate reader according to the manufacturer's instructions by using the blank wells.

If you have any particular questions concerning this kit, please do not hesitate to contact us under the following numbers:

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CTS reagents are provided exclusively for investigational use within the Collaborative Transplant Study