Human CLEC5A ELISA Kit

Instructions for Use | Cat. No.: ARE6651

1. Content

Component	CAT	Volume
CP (Coated Plate)	EK2175-CAP	96 well
ST (Standard)	EK2175-ST	10 ng/tube x 2
DA (Detect Antibody)	EK2175-DA	100 µl (100×)
ABC (Avidin-Biotin-Peroxidase Complex)	AR1103	100 µl (100×)
SD (Sample Diluent)	AR1106-1	30 ml
AD (Antibody Diluent)	AR1106-2	12 ml
ABCD (ABC Diluent)	AR1106-3	12 ml
TMB (TMB Substrate)	AR1104	10 ml
SS (Stop Solution)	AR1105	10 ml
WB (Wash Buffer 25×)	AR1106-5	20 ml
SF (Adhesive Cover)	PLA-SEA	4 pieces

NOTE: The unopened kit can be stored at 2-8°C for 6 months or at -20°C for 1 year. After opening, please use the kit as soon as possible; opened components can be stored at 2-8°C for one month.

2. Reagent Preparation

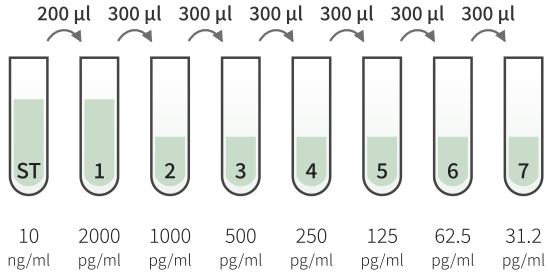
Washing Buffer (1×) Preparation

Dilute 12 ml of the 25× Washing Buffer with 288 ml of deionized or distilled water to prepare the 1× Washing Buffer. Mix thoroughly. Prepare the working solution within 1 hour of use.

Standard Curve Preparation

Reconstitute one tube of the lyophilized recombinant human CLEC5A Standard with 1 ml of Sample Diluent and let it stand for at least 10 minutes, then vortex to mix thoroughly to obtain the 10,000 pg/ml standard stock. Use within 2 hours of preparation.

Add 800 µl of Sample Diluent and 200 µl of the 10,000 pg/ml standard into tube #1 to prepare the 2000 pg/ml high standard. Add 300 µl of Sample Diluent into tubes #2 to #7. Transfer 300 µl from tube #1 into tube #2, mix thoroughly, and continue the 1:2 serial dilution from tube #2 to tube #7. Sample Diluent serves as the zero standard (0 pg/ml).



Biotinylated Antibody Working Solution

Dilute the Biotinylated Antibody 1:100 with Antibody Diluent (1 µl of antibody + 99 µl of Antibody Diluent) and mix gently. Prepare the working solution within 2 hours of use. Calculate the total volume at 100 µl per well and prepare 100-200 µl in excess.

ABC Working Solution

Dilute the Avidin-Biotin-Peroxidase Complex (ABC) 1:100 with ABC Diluent (1 µl of ABC + 99 µl of ABC Diluent) and mix gently. Prepare the working solution within 1 hour of use. Calculate the total volume at 100 µl per well and prepare 100-200 µl in excess.

3. Assay Procedure

Bring all reagents and samples to room temperature before use. Mix thoroughly when diluting reagents or samples. A standard curve must be run with each assay.

1. Determine the number of pre-coated wells required. Total wells = number of samples + 9 (8 standard wells and 1 TMB blank well); double the number for duplicate measurements. Reseal the unused strips and store them at 4°C.

2. Add 100 µl of each serially diluted standard (S1-S7) into 7 wells and 100 µl of **SD** (Sample Diluent) into one well as the zero standard. Add 100 µl of each sample into the remaining wells. Leave the TMB blank well empty. Cover with an **SF** (Adhesive Cover) and incubate at 37°C for 90 minutes.

3. Aspirate or decant the liquid from the wells and blot the plate against absorbent paper. Do not wash.

4. Add 100 µl of **DA** (Detect Antibody) Working Solution into each well, except the TMB blank well. Cover with an SF (Adhesive Cover) and incubate at 37°C for 60 minutes.

5. Wash the plate 3 times with 1× **WB** (Washing Buffer), soaking for 60 seconds each time (at least 300 µl per well).

6. Add 100 µl of **ABC** Working Solution into each well, except the TMB blank well. Cover with an SF (Adhesive Cover) and incubate at 37°C for 30 minutes. Equilibrate the **TMB** (TMB Substrate) at 37°C for 30 minutes at the same time.

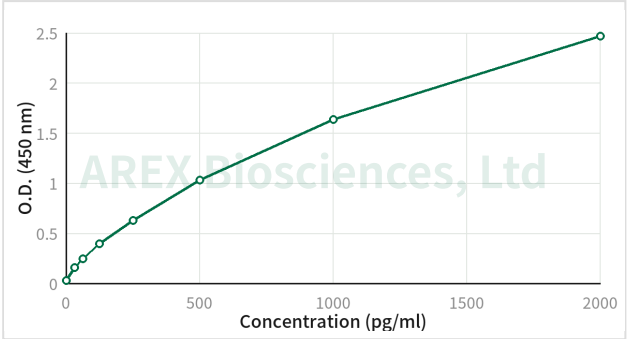
7. Wash the plate 5 times with 1× WB (Washing Buffer), soaking for 90 seconds each time (at least 300 µl per well).

8. Add 90 µl of **TMB** (TMB Substrate) into each well and incubate at 37°C in the dark for 15 minutes. The TMB blank well receives only TMB Substrate and **SS** (Stop Solution). The development time may be adjusted according to the actual color development but must not exceed 30 minutes.

9. Add 100 µl of **SS** (Stop Solution) into each well in the same order as the TMB Substrate. The color changes from blue to yellow immediately.

10. Read the optical density immediately at 450 nm with a microplate reader.

4. Typical Data



Concentration (pg/ml)	O.D.
0	0.027
31.2	0.158
62.5	0.244
125	0.395
250	0.629
500	1.029
1000	1.635
2000	2.464

5. Sensitivity

The minimum detectable dose (MDD) of Human CLEC5A is typically less than 10 pg/ml.