

KIT COMPONENTS

Item	Description	Storage Conditions
Human CC10 Microtiter Plate	One 96 well microtiter plate coated with anti-human CC10 antibody	4°C; up to 6 months
Megatiter Assay Block	96 well block for mixing of assay solutions prior to addition to the plate	n/a
Calibrator Stock Solution	100 µl of rhCC10, 50 µg/ml	4°C; up to 6 months (Do not freeze)
Conjugate Stock Solution	30 µl of human CC10-HRP conjugate	-20°C; up to 6 months (Freeze upon receipt)
Assay Buffer Concentrate	12 ml of 10X concentrate	4°C; up to 6 months
Wash Buffer	120 ml of 1X stock	4°C; up to 6 months
Color Development Solution	12 ml of 3,3',5,5'-Tetramethylbenzidine (TMB)	4°C; up to 6 months
Human Serum Control	200 µl undiluted human serum (pre-tested negative for standard panel of infectious diseases); [CC10] range 50-60 ng/ml	-20°C; up to 6 months (Freeze upon receipt)

STORAGE NOTES

The conjugate stock and human serum control must be stored at -20°C. The rest of the kit is stored at 4°C. Diluted conjugate cannot be stored. Discard after each assay. Diluted Assay Buffer should be used within 24 hours. Wash Buffer can be stored at room temperature for about 6 weeks or at 4°C for 6 months.

OTHER MATERIALS NOT SUPPLIED IN KIT

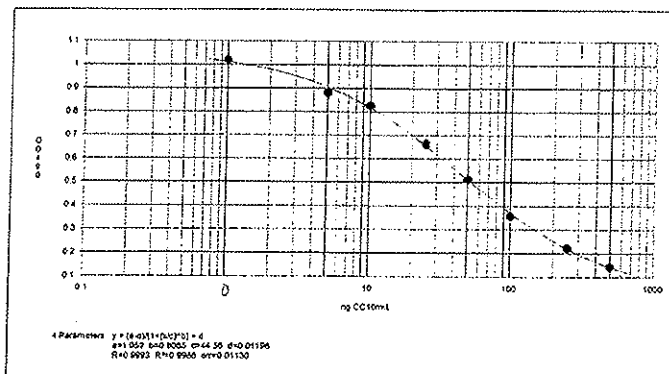
1M HCl
Microplate reader capable of measuring absorbance at 450 nm.
Multichannel pipette, automated microplate washer and/or squirt Bottle.
Deionized or distilled water

ASSAY DESCRIPTION

In this competitive ELISA format, an anti-CC10 antibody is used as the capture reagent for CC10 in the sample. A conjugate of horse radish peroxidase (HRP) to recombinant human CC10 is captured by the anti-CC10 antibody coated in the wells and generates a signal (A₄₅₀) proportional to the amount of CC10-HRP conjugate bound. The CC10-HRP conjugate is pre-mixed with the sample to be assayed (which may be pre-diluted with PBS, if necessary). Typically, two different dilutions (1:2-3 and 1:10) of each sample are run in duplicate, using between 25-110 µl of sample. The assay thus measures a decrease in signal as the CC10 in the sample competes the CC10-HRP conjugate for binding sites (see figure). A standard curve, using carefully prepared CC10 calibrators, is always run in duplicate with each set of samples to assess reproducibility and accurately quantitate CC10 in samples. X-axis is CC10 concentration in nanograms/ml and Y-axis is absorbance at 450 nm. Greatest accuracy occurs in the linear range between 10-100 ng/ml. Samples measuring higher than 200 ng/ml should be diluted and retested. Coefficients of Variation (CV's) are typically less than 15%.

Sensitivity and Specificity. The competitive CC10 ELISA is sensitive to about 5 ng/ml and has been used to measure human CC10 in a number of different types of background matrices from humans and other species. Normal plasma levels of endogenous CC10 in healthy humans are typically between 10-100 ng/ml. Under the conditions used in the ELISA, no cross-reactivity has been observed with non-human CC10 in plasma samples from pigs, rats, mice, sheep, and rabbits. Native CC10 in BAL of healthy individuals ranges from 1-25 microgram/ml, and up to 0.5-1.0 milligram/ml in induced sputum. Some cross-reactivity has been observed in BAL samples as measured by relatively high "background" (10-25 ng/ml). It may not be possible to distinguish between human CC10 and native CC10 in other primates.

STANDARD CURVE



A competitive ELISA for human CC10 was used in the following publications:

1. Levine CR, et al. *Pediatr Res.* 58(1):15-21 (2005).
2. Chandra S, et al. *Pediatr Res.* 54(4):509-15 (2003).
3. Miller TL, et al. *Pediatr Crit Care Med* 6:696-706 (2005).
4. Nosratabadi AR, et al. *Exp Lung Res.* 29:455-73 (2003).
5. Miller TL, et al. *Biology of the Neonate* 89:159-170 (2006).
6. Shashikant BN, et al. *J Appl Physiol.* 99(6):2204-11 (2005).

Warning: This product is not intended to be used in the diagnosis or treatment of any disease or condition. Not for human use

INSTRUCTIONS

PREPARE TO RUN THE ASSAY

A. Assay buffer is supplied as a 10X concentrate. It may be necessary to warm the 10X assay buffer for a few minutes at 37°C to bring it fully into solution. Dilute to a 1X working solution with deionized or distilled water. Dilute only what you need for each assay.

B. Preparation of calibrators – It is essential to create a standard curve using calibrators diluted from the concentrated calibrator stock solution. Calibrators of 0, 5, 10, 25, 50, 100, 250 and 500 ng/ml are recommended. The standard curve is used to determine the concentrations of all other samples. Improper dilution of calibrators will result in inaccurate results. The following dilution scheme for calibrators is recommended. Run each calibrator in duplicate.

Dilution Scheme
(Sufficient to run duplicate wells for each sample)

Calibrator concentration ng/ml	Assay Buffer	Volume and Source of Calibrator
500	990 µl	10 µl calibrator stock supplied
250	200 µl	200 µl of 500 ng/ml
100	300 µl	200 µl of 250 ng/ml
50	200 µl	200 µl of 100 ng/ml
25	200 µl	200 µl of 50 ng/ml
10	300 µl	200 µl of 25 ng/ml
5	200 µl	200 µl of 10 ng/ml
0	200 µl	0 (control PBS)

C. Preparation of conjugate- Calculate the amount of diluted conjugate needed using the following calculation.

$$\text{Volume diluted conjugate} = [(\# \text{ calibrators} + \# \text{ samples} + \# \text{ controls}) \times 0.11 \text{ ml}] + 0.5 \text{ ml}$$

Serial dilution of the conjugate:

1. Make a 1:100 dilution of the conjugate in 1 ml of assay buffer.
Add 990 µl assay buffer to 10 µl concentrated conjugate
2. Make a 1:200 dilution in assay buffer using the volume calculated.
Add 5 µl of the 1:100 dilution for each 1 ml of conjugate needed.
3. Discard all unused diluted conjugate solutions when the assay is complete.

D. Preparation of control sera (optional)- The human serum control (from a normal individual) contains between 10-100 ng/ml of native CC10. Serum controls may be run undiluted or may be diluted 1:2.

E. Preparation of samples- Samples should be free of particulates which can interfere with reading the results of the assay. Centrifuge or filter samples as needed.

ASSAY PROCEDURE

Bring all reagents to room temperature before use.

1. Plan which wells will contain which samples and calibrators. Create a “map” of the samples using the table provided. Use corresponding wells on the assay block and microtiter plate for each sample and calibrator.
2. Add 110 µl of each calibrator, sample, or control to appropriate wells of the assay block.
3. Add 110 µl of diluted conjugate to each calibrator, sample, and control in the assay block.
4. Mix samples in all of the wells by pipeting each mixture up and down 5-7 times.
5. Pipette 100 µl from the assay block to each of 2 wells in the 96 well microtiter plate so that each calibrator, sample, and control is duplicated.
6. Incubate the plate with gentle rotation or rocking for 60 min. Do not incubate longer than 75 minutes.
7. Aspirate each well and wash with 300 µl of Wash Buffer for a total of 3 washes. Use of an automated plate washer is preferred, however, a multichannel pipette or squirt bottle may be used (very gently), provided care is taken to avoid dislodging antibody complexes from the plate wells. Invert the plate and blot it on clean paper towels.
8. Add 100 µl of Color Development Solution to each well. Incubate for 10 minutes at room temperature. As the color develops, tap the plate gently a few times to mix. Do not incubate longer than 15 minutes.
9. Stop the reaction with 100 µl of 1M HCl. This will stop the color development and turn the substrate yellow.
10. Read in microtiter plate reader at a wavelength of 450 nm. Reaction must be read within an hour.