ELISA antibody pair

Technical Data Sheet

10-plate and 20-plate format

For research use only.
Not for use in diagnostic or therapeutic procedures.

Introduction

The usefulness of sandwich ELISAs (enzyme-linked immunosorbent assays) in cytokine biology is evident from the many reports published on this subject. The assay requires two antibodies (either mono- or polyclonal antibodies) that bind with high affinity to different sites on the cytokine molecule. One of the antibodies is immobilized to the wells of a 96-well microtiter plate. This so-called capture or coating antibody functions to selectively immobilize the cytokine from crude protein preparations. The second antibody (detection antibody) is labeled with biotin and binds to a different site on the cytokine molecule. Biotin allows the antibody to interact with streptavidin molecules. By using HRP (horseradish peroxidase)-labeled streptavidin, the cytokine can now quantitatively be determined by enzymatic conversion of a HRP-specific substrate to a colored product. Since many biotin molecules can be coupled to the detector antibody, several HRP molecules are bound. The enzyme activity is subsequently further increased by using complexes (polymers) of HRP-streptavidin. This results in an extreme sensitive assay with a detection limit within the low picogram range (~5 pg/ml).

Contents

10-plate format

- 2 vials with anti-cytokine coating antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 2 vials with biotinylated anti-cytokine detection antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 5 vials with cytokine standards supplied in lyophilized form.
- 2 vials with SPP conjugate (Streptavidin-HRP) supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.

20-plate format

- 4 vials with anti-cytokine coating antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 4 vials with biotinylated anti-cytokine detection antibody supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.
- 10 vials with cytokine standards supplied in lyophilized form.
- 4 vials with SPP conjugate (Streptavidin-HRP) supplied in lyophilized form; each vial contains sufficient material for five 96-well ELISA plates.

Hazard information

Components of the antibody pair are not classified as dangerous according to Regulation (EC) no. 1272/2008 and Directive 67/548/EC or 1999/45/EC and their amendments.

Please find the Material Safety Data Sheet on www.aniara.com

ANIARA 7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com www.aniara.com

This Technical Data Sheet applies for the following U-CyTech ELISA antibody pairs

(please find below the catalogue number of the ELISA antibody pair)

Analyte	Species				
	Human	Old World Monkey	New World Monkey	Mouse	Rat
IFN-γ	ACT740-10 (10-plate) ACT740-20 (20-plate)	ACT710-10 (10-plate) ACT710-20 (20-plate)	ACT770-10 (10-plate) ACT770-20 (20-plate)	ACT755-10 (10-plate) ACT755-20 (20-plate)	ACT700-10 (10-plate) ACT700-20 (20-plate)
IL-1β	ACT751-10 (10-plate) ACT751-20 (20-plate)	ACT708-10 (10-plate) ACT708-20 (20-plate)			
IL-2	ACT741-10 (10-plate) ACT741-20 (20-plate)	ACT711-10 (10-plate) ACT711-20 (20-plate)		ACT762-10 (10-plate) ACT762-20 (20-plate)	
IL-4	ACT742-10 (10-plate) ACT742-20 (20-plate)	CT712-10 (10-plate) ACT712-20 (20-plate)		ACT757-10 (10-plate) ACT757-20 (20-plate)	ACT702-10 (10-plate) ACT702-20 (20-plate)
IL-5	ACT743-10 (10-plate) ACT743-20 (20-plate)	AACT713-10 (10-plate) ACT713-20 (20-plate)		ACT760-10 (10-plate) ACT760-20 (20-plate)	
IL-6	ACT744-10 (10-plate) ACT744-20 (20-plate)	ACT714-10 (10-plate) ACT714-20 (20-plate)		ACT763-10 (10-plate) ACT763-20 (20-plate)	
IL-8	ACT748-10 (10-plate) ACT748-20 (20-plate)	ACT718-10 (10-plate) ACT718-20 (20-plate)			
IL-10	ACT745-10 (10-plate) ACT745-20 (20-plate)	ACT715-10 (10-plate) ACT715-20 (20-plate)		ACT758-10 (10-plate) ACT758-20 (20-plate)	
IL-12/23p40		ACT719-10 (10-plate) ACT719-20 (20-plate)			
IL-12p70	ACT750-10 (10-plate) ACT750-20 (20-plate)				
IL-13	ACT746-10 (10-plate) ACT746-20 (20-plate)	ACT716-10 (10-plate) ACT716-20 (20-plate)	ACT771-10 (10-plate) ACT771-20 (20-plate)		
IL-17	ACT566-10 (10-plate) ACT566-20 (20-plate)	ACT551-10 (10-plate) ACT551-20 (20-plate)			
IL-23	ACT567-10 (10-plate) ACT567-20 (20-plate)	ACT552-10 (10-plate) ACT552-20 (20-plate)			
G-CSF	ACT737-10 (10-plate) ACT737-20 (20-plate)				
GM-CSF	ACT739-10 (10-plate) ACT739-20 (20-plate)	ACT709-10 (10-plate) ACT709-20 (20-plate)			
Granzyme B	ACT752-10 (10-plate) ACT752-20 (20-plate)				
Perforin	ACT753-10 (10-plate) ACT753-20 (20-plate)	ACT720-10 (10-plate) ACT720-20 (20-plate)			
TNF-α	ACT747-10 (10-plate) ACT747-20 (20-plate)	ACT717-10 (10-plate) ACT717-20 (20-plate)	ACT772-10 (10-plate) ACT772-20 (20-plate)	ACT761-10 (10-plate) ACT761-20 (20-plate)	ACT704-10 (10-plate) ACT704-20 (20-plate)

ANIARA 7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com www.aniara.com

Required materials and reagents

- PB stock: dissolve 96.0 g $Na_2HPO_4.2H_2O$ plus 17.5 g KH_2PO_4 in 1.0 L distilled water and adjust pH to 7.4
- Sterile distilled water
- 2 M H_2SO_4
- Bovine serum albumin (BSA; ELISA grade)
- 96-well ELISA plates. Plates from Greiner Bio-one are recommended (Greiner Bio-one cat.no.655092 or U-CyTech cat.no. CT361)
- Adhesive cover slips (Greiner Bio-one cat.no. 676001)
- Pipetting devices for the accurate delivery of volumes required for assay performance
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Reading device for microtiter plates set at 370, 450 or 655 nm
- Tween-20 detergent
- Coloring system (e.g. tetramethylbenzidine [TMB]). Reagents from U-CyTech (cat.no. CT367 [TMB tablets] and CT369 [Substrate buffer capsules]) are recommended.

Working solutions

- PBS: 10 ml PB stock solution and 8.8 g NaCl added to 1.0 L distilled water
- PBST (= wash buffer): 0.5 ml Tween-20 added to 1 L PBS
- PBST-B : PBST supplemented with 0.5% (w/v) BSA
- Blocking buffer: PBS supplemented with 1% (w/v) BSA

ANIARA 7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com
www.aniara.com

ELISA protocol

Coating antibody

- Reconstitute the contents of the vial in 250 µl distilled water and dilute 100-fold in PBS.
- Bring 50 µl of diluted coating antibodies in the wells of an ELISA plate and fill up to 100 µl with PBS.
- Seal the plate with an adhesive cover slip and incubate overnight at 4°C.

Blocking

- Remove coating antibody solution and wash the wells at least 6 times with PBST.
- Add 200 µl blocking buffer to each well.
- Seal the plate with an adhesive cover slip and incubate 1 h at 37°C or 2 h at room temperature.

Samples and standards

- Dilute samples and standards in PBST-B (see 'Cytokine standards').
- Remove blocking buffer by a vigorous 'shake-out' action but do not wash.
- Add 100 µl of diluted standards and samples to each well.
- Seal the plate and incubate for 2 h at 37°C.

Detection antibody

- Reconstitute the contents of the vial in 500 µl distilled water and dilute 100-fold in PBST-B.
- Remove standards and samples and wash the wells at least 6 times with PBST.
- Add 100 µl of diluted detector antibodies to each well.
- Seal the plate and incubate 1 h at 37°C or overnight at 4°C.

Enzyme conjugate

- Reconstitute the contents of the vial with SPP in 500 µl distilled water and dilute 100-fold in PBST-B.
- Remove detector antibody solution and wash the wells at least 6 times with PBST.
- Add 100 µl of diluted SPP conjugate to each well.
- Seal the plate and incubate 1 h at 37°C or overnight at 4°C.

Substrate

- The substrate for SPP should be prepared as follows: dissolve one TMB tablet (U-CyTech cat.no. CT367 or Sigma cat.no. T-5525) in 1.0 ml dimethylsulfoxide (DMSO) and add this to 10 ml substrate buffer (Phosphate-Citrate buffer containing sodium perborate [U-CyTech cat.no. CT369 or Sigma cat.no. P-4922]).

ANIARA 7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com www.aniara.com

- Remove conjugate solution and wash the wells at least 6 times with PBST.
- Immediately dispense 100 µl substrate solution into each well and incubate at room temperature (15-30 minutes). In case of a positive reaction, the colorless solution will become blue that can be read at 370 nm or 655 nm. The reaction can be stopped by adding 50 µl 2M H_2SO_4 (resulting in a yellow color that can be read at 450 nm).

Cytokine standards

For maximum recovery, the vial with lyophilized cytokine standards should be reconstituted in distilled water (volume indicated on the vial) and allowed to stand for 1 minute at room temperature. Thereafter, the reconstituted cytokine standard should be used immediately (preferentially within one hour). For measuring cytokines in cell culture supernatant, both test samples and standards should be diluted in PBST-B. However, when measuring cytokines in serum or plasma, the standard diluent should preferentially be control serum or plasma originating from the same species.

Storage antibodies and standards

The vials with lyophilized coating antibodies and biotinylated detector antibodies can be safely stored in a refrigerator for a defined length of time (expiry date indicated on the vial). After reconstitution, the antibodies remain fully active for minimal 6 months at 4°C when kept sterile. However, it is strongly recommended to divide the reconstituted antibody solutions into small aliquots for single use. These aliquots should be stored at ≤ -20°C. Under these conditions the antibodies are stable for at least one year.

The vials with lyophilized cytokine standard can be safely stored in a refrigerator for one year. Use the vials with cytokine standard only once.

The vials with lyophilized SPP conjugate can be safely stored at ≤ -20°C for a defined length of time (expiry date indicated on the vial). After reconstitution, the conjugate can best be divided into small aliquots for single use. These aliquots should be stored at ≤ -20°C. Under these conditions the conjugate is stable for at least one year.

Directions for washing

Incomplete washing will adversely affect the assay. All washing must be performed with wash buffer (PBST).

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into each well. After aspiration, fill the wells with at least 300 µl wash buffer, then aspirate the liquid. After washing, the plate is inverted and tapped dry on absorbent paper.

Alternatively, the wash buffer may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After washing, the plate is inverted and tapped dry on absorbent paper.

If using an automated washing device, the operating instructions should carefully be followed.

ANIARA 7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com
www.aniara.com

Trouble shooting

Poor consistency of replicates can be overcome by increasing the stringency of washes particularly after the incubation step with detector antibody.

High values of the blank control (optical density > 0.2) can be overcome by shortening the incubation time with the substrate solution or is caused by improper washing procedures.

Inconsistent replicates may be due to cross-contamination of wells by improper pipetting procedures.

If no signal is observed in the wells with the standards,

- try a new vial with cytokine standard
- check whether the substrate solution was properly prepared (pH should be between 5.0 and 5.5)
- verify whether the antibodies, SPP conjugate and standard preparations were properly diluted
- verify whether the SPP conjugate was properly stored (storage at room temperature can lead to a significant loss of SPP activity and consequently low OD readings)

Avoid sodium azide in wash buffers and diluents, as this is an inhibitor of peroxidase activity.

ANIARA 7768 Service Center Drive • West Chester OH 45069
Phone: 513.770.1991 Toll Free: 866.783.3797 Fax: 513.573.9241 Email: info@aniara.com
www.aniara.com