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Human Granzyme B Matched Antibody Pair for ELISA

Catalog No: CKH330 **Size:** 10 x 96 tests **Lot:** TBD **Expiration Date:** TBD

NOTE: this is a sample protocol which is subject to variation by Lot Number. Refer to the protocol inserted in your package for the current lot number specifications and expiration date or contact our technical support at tech@cellsciences.com

Specificity:	Native and recombinant human Granzyme B
Sensitivity:	25 pg/mL
Range:	0.5 – 100 pg/mL
Sample Type:	Cell supernatants, buffered solutions, serum, plasma samples and other bodily fluids.
Cross-Reactivity:	None observed for any protein tested

INTRODUCTION

Cell Sciences® Granzyme B Matched Antibody Pair is intended for use in a "do it yourself" solid phase sandwich ELISA for the *in vitro* qualitative and quantitative determination of human Granzyme B in biological fluids such as cell culture supernatant, plasma, or serum. A capture antibody is provided for coating microtiter plates. Standards and samples are added to coated plates and the coating antibody selectively immobilizes the analyte from the test material. The detection antibody, which is labeled with biotin, binds to another epitope of the analyte. Streptavidin-HRP (SPP) conjugate is added and binds to the biotinylated detection antibody. A chromogenic substrate (such as TMB) is introduced and the subsequent reaction with the HRP produces a colored product, of which the intensity is related to the amount of analyte in the sample. OD values for each standard are plotted against expected concentration to form a standard curve. This standard curve can then be used to accurately determine the concentration of analyte in any sample tested.

REAGENTS PROVIDED:

CKH330-A	Coating antibody – monoclonal antibody to human Granzyme B	**2 vials**
CKH330-B	Detection antibody – biotinylated monoclonal antibody to human Granzyme B	**2 vials**
CKH330-C	Standards – native human Granzyme B	**5 vials**
CKH330-D	SSP Conjugate (Streptavidin-HRP)	**2 vials**

Coating antibodies CKH330-A

Product: Monoclonal antibody to human granzyme B
Isotype: Mouse IgG_1
Production: *In vitro* using serum free medium
Purification: Ammonium sulfate precipitation and affinity chromatography
Formulation: Prior to lyophilization: 250 µL PBS, 125 mM trehalose
Preparation: Add 250 µL distilled water into the vial and dilute 100 times in PBS. The content of one vial is sufficient for five 96-well ELISA plates (480 determinations; 50 µL/well).

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Detection antibodies CKH330-B

Product:	Biotinylated monoclonal antibody to human granzyme B
Isotype:	Mouse IgG1
Production:	*In vitro* using serum free medium
Purification:	Ammonium sulfate precipitation and affinity chromatography
Labeling:	With Biotin-7-NHS (N-hydroxysuccinimide)
Formulation:	Prior to lyophilization: 500 µL PBS, 1% BSA, 125 mM trehalose
Preparation:	Add 500 µL distilled water into the vial and dilute 100 times in PBS, 0.5% BSA, 0.05% Tween-20. The content of one vial is sufficient for five 96-well ELISA plates (480 determinations; 100 µL/well).

Standards CKH330-C

Product:	Natural human granzyme B (cell-culture derived)
Application:	Standard in an ELISA system
Preparation:	Dissolve the contents of one vial by injection of 500 µL distilled water into the vial. Use immediately.

Conjugate CKH330-D

Product:	SPP conjugate (Streptavidin-HRP)
Application:	Add 500 µL distilled water into the vial and dilute 100 times in PBS, 0.5% BSA, 0.05% Tween-20. The content of one vial is sufficient for five 96-well ELISA plates (480 determinations; 100 µL/well).

MATERIALS/ REAGENTS REQUIRED BUT NOT PROVIDED

- PBS (pH 7.4).
- Sterile distilled water
- Bovine serum albumin (BSA; ELISA grade)
- Tween-20
- Substrate Solution e.g. 3,3′,5,5′-Tetramethylbenzidine (TMB) and Stop solution (H2SO4). Ready-to-use TMB substrate solution is recommended, in combination with 0.18 M H_2SO_4 as Stop solution.
- Microtiter plates
- Adhesive plate covers
- Pipetting devices
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
- Reading device for microtiter plates (which fulfills the requirements of the applied substrate).

PREPARATION OF SOLUTIONS AND REAGENTS

- PBS
- Wash buffer: 500µL Tween-20 added to 1 L PBS
- Blocking buffer: PBS supplemented with 1% (w/v) BSA

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- Dilution buffer: PBS supplemented with 0.5% (w/v) BSA and 0.05% (w/v) Tween-20
- Coating antibody (CKH330-A), Biotinylated detection antibody (CKH330-B), SPP conjugate (CKH330-D): Reconstitute the lyophilized product by injecting the appropriate volume (indicated on the vial and Data sheet) of sterile distilled water into the vial. Mix the solution gently for approximately 15 seconds. Allow to stand for 5 minutes at room temperature. Avoid vigorous shaking. Dilute 100-fold with Dilution Buffer.
- Standard (CKH330-C): Reconstitute the lyophilized standard by adding 500 µL of sterile distilled water into the vial. Mix the solution gently for approximately 15 seconds and allow it to stand for 1 minute at room temperature. Avoid vigorous shaking. Dilute immediately (preferentially within one hour) in Dilution buffer to the desired concentrations to be used in the standard curve range. In general, when TMB substrate solution is used; the linear portion of the standard curve falls within the range of 0.5 to 100 pg/mL.

STORAGE INSTRUCTIONS

The vials with lyophilized coating and biotinylated detection antibody can be safely stored in a refrigerator until expiration date (**TBD**). After reconstitution, the antibodies are stable for at least 1 year at 4 °C when kept sterile. However, it is recommended to aliquot the reconstituted antibody solutions into small aliquots for storage at ≤-20 °C. The vials with lyophilized cytokine standard can be safely stored at 4 °C until the expiration date. These vials are for single use only. The vial of lyophilized SPP conjugate is stable until expiration date when stored in the dark at ≤-20 °C. After reconstitution, the reagent is stable for 2 months under sterile conditions and in the dark at 4 °C. Divide solution into small aliquots for single use, and store in the dark at ≤ -20 °C.

PREPARATION OF SAMPLES

- Specimens should be clear, non-hemolyzed and non-lipemic. Excessive hemolysis and the presence of large clots or microbial growth in the sample may interfere with the performance of the test.
- Dilute samples in Dilution buffer (at least 1:1).
- The diluent for the standard and blank control should preferentially be control serum or plasma originating from the same species. For measuring cytokines in cell culture supernatant, samples should be diluted in Dilution buffer.
- Avoid repeated freeze-thaw cycles of samples.

WASHING PROCEDURE

- Incomplete washing of the wells will adversely affect the assay.
- Recommended washing procedure: Completely empty the wells and then fill with at least 250 µL of Wash Buffer. Let plate soak for 10-20 seconds, then empty the wells. Repeat these steps at least six times. After washing, invert the plate and tap dry on absorbent paper.

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ASSAY PROTOCOL

Assay Step	Details
1. Addition	Add 50 µL of diluted coating antibody (CKH330-A) in the wells of an ELISA plate and fill up to 100 µL with PBS.
2. Incubation	Seal the plate with an adhesive cover slip and incubate overnight at 4 °C.
3. Wash	Remove coating antibody solution and wash the wells at least 6 times with Wash buffer
4. Addition	Add 200 µL Blocking buffer to each well.
5. Incubation	Seal the plate with an adhesive cover slip and incubate 1 hour at 37 °C
6. Wash	Empty the wells by flicking the plate & gently tapping on absorbent paper. Do not wash.
7. Addition	Add 100 µL of diluted standards (CKH330-C) or samples to appropriate wells.
8. Incubation	Seal the plate and incubate for 2 hours at 37 °C (or alternatively overnight at 4 °C)
9. Wash	Remove standards and samples and wash the wells at least 6 times with Wash buffer.
10. Addition	Add 100 µL of diluted detector antibody (CKH330-B) to each well.
11. Incubation	Seal the plate and incubate 1 hour at 37 °C.
12. Wash	Remove detection antibody solution and wash the wells at least 6 times with Wash buffer.
13. Addition	Add 100 µL of diluted SPP conjugate (CKH330-D) to each well.
14. Incubation	Seal the plate and incubate 1 hour at 37 °C.
15. Wash	Remove conjugate solution and wash the wells at least 6 times with Wash buffer
16. Addition	Add 100 µL of ready-to-use HRP substrate (e.g. TMB) to each well
17. Development	Incubate the plate for 5-15 minutes monitoring visually throughout the incubation period to assess sufficient color development
18. Addition	Stop the color development reaction. If using TMB, add 100 µL 0.18 M H_2SO_4.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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