# Human Granzyme B ELISPOT Ready-SET-Go!®

**Catalog Number:** 88-8399
**Also known as:** GrzB, GrB
**RUO: For Research Use Only. Not for use in diagnostic procedures.**

## Product Information

**Contents:** Human Granzyme B ELISPOT Ready-SET-Go!®
REF **Catalog Number:** 88-8399

**Temperature Limitation:** Store at 2-8°C.
LOT **Batch Code:** Refer to vial
**Use By:** Refer to vial
**Caution, contains Azide**

## Description

One of the major mechanisms of cell-mediated cytotoxicity involves exocytosis of cytoplasmic granules from the effector toward the target cell. The granules contain a number of proteins, including the pore-forming protein perforin and a family of serine proteases called granzymes, including Granzyme B. Granzyme B is present mainly in the granules of CD8+ CTL and natural killer (NK) cells and mediates the lethal hit that kills virus infected and tumorigenic cells. Measurement of release of Granzyme B in response to the appropriate target is useful for evaluating cell-mediated cytotoxicity. The Granzyme B ELISPOT assay is a valuable non-radioactive alternative to the 51Cr-release killing cell assays for measuring antigen-specific CTL cytotoxicity.

This Granzyme B ELISPOT Ready-SET-Go! reagent set contains the necessary reagents for performing enzyme linked immunosorbent spot (ELISPOT) assays for high resolution frequency analysis of Granzyme B-secreting cells. This ELISPOT reagent set is pre-titrated for optimal spot development.

## Components

**Capture Antibody**. Pre-titrated, Functional Grade (low endotoxin) purified antibody
**Detection Antibody**. Pre-titrated, biotin-conjugated antibody
**ELISA/ELISPOT Coating Buffer**. This Ready-Set-Go! ELISPOT Set may contain ELISA/ELISPOT Coating Buffer Powder (Reconstitute to 1L with dH20 and filter (0.22 uM)) or 10X PBS ELISPOT Coating Buffer (Dilute 1 part 10X Buffer into 9 parts dH20 and filter with 0.22 uM).
**Assay Diluent**. 5X concentrated
**Detection enzyme**. Pre-titrated Avidin-HRP
**Certificate of Analysis**. Lot-specific instructions for dilution of antibodies and enzyme

## References

Shafer-Weaver, K., et al. 2003. The Granzyme B ELISPOT assay: an alternative to the 51Cr-release assay for monitoring cell-mediated cytotoxicity. J. Translational Med. 1: 14.
Rininsland, F., et al. 2000. Granzyme B ELISPOT assay for ex vivo measurements of T cell immunity. J Immunol Meth. 240:143-155.

## Related Products

11-8898 Anti-Mouse Granzyme B FITC (NGZB)
12-8899 Anti-Human Granzyme B PE (GB11)
25-8898 Anti-Mouse Granzyme B PE-Cy7 (NGZB)
88-8022 Mouse Granzyme B ELISA Ready-SET-Go!®


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# ELISPOT Set Protocol

## Research Use Only

### Additional Materials Needed

- 96-Well PVDF Membrane ELISPOT Plates (Millipore, Cat. No. MAIPS4510)
- AEC (3-amino-9-ethyl carbazole) Substrate (Sigma, Cat. No. A-5754)
- Distilled water
- ELISPOT Wash Buffer: 1X PBS, with 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, cat 00-0400)

### Instruments

- Pipettes and pipettors
- Refrigerator
- Incubator
- Laminar Flow Hood
- Plate Washer: Wash bottle or automated wash machine
- ELISPOT plate reader or dissecting microscope for visual inspection

### Experiment Duration

- 1 overnight antibody incubation
- 1-2 day cell activation
- 3-5 hr washing, antibody incubation, color development

### ELISPOT Method

Aseptic Procedure: (Use sterile buffers and aseptic conditions; use laminar flow hood for procedures.)

1. Dilute Functional Grade purified capture antibody in sterile ELISPOT Coating Buffer, as noted on Certificate of Analysis which is included with the reagent set. Coat ELISPOT plate with 100 µl/well of capture antibody solution. Incubate at 4°C overnight.
2. Decant or aspirate coating antibody from plate.
3. Wash plates 2 times with 200 µl/well sterile ELISPOT Coating Buffer. Decant.
4. Block plate with 200 µl/well of complete RPMI-1640 at room temperature for 1 hour. Decant or aspirate plate.
5. Aliquot mitogen, antigen or controls diluted in complete RPMI-1640 medium to appropriate wells at 100 µl/well. Aliquot cells at desired densities (e.g., $1x10^5$/ml - $2x10^6$/ml) at 100 µl/well and incubate at 37°C, 5% $CO_2$ humidified incubator for 24-48 hours.
   ***Note**: Kinetics and cell densities vary with target cytokine, treatment, and cell type and must be empirically determined. See references. Cells can be diluted in a sterile tissue culture plate starting at $2x10^6$/well in triplicate wells with a series of 1:3 or 1:4 serial dilutions down the plate, and then transferred to the ELISPOT plate.*



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Non-Aseptic Procedure:

1. Decant cells and medium from plates. Wash plate 3 times with ELISPOT Wash Buffer.
2. Dilute biotinylated detection antibody in Assay Diluent according to instructions on the Certificate of Analysis provided with the reagent set. Add 100 µl/well to plate microwells and incubate at room temperature for 2 hr (or 4°C overnight).
3. Decant antibody solution. Wash 4 times with ELISPOT Wash Buffer. Allow wells to soak for 1 minute for each wash.
4. Dilute Avidin-HRP reagent in Assay Diluent according to instructions on the Certificate of Analysis provided with the reagent set. Add 100 µl/well of Avidin-HRP and incubate at room temperature for 45 minutes.
5. Decant Avidin-HRP solution. Wash plate 3 times with ELISPOT Wash Buffer, and then 2 times with 1X PBS (no Tween-20).
6. Add 100 µl/well of freshly-prepared AEC Substrate Solution and develop at room temperature for 10-60 minutes; monitor development of spots.
7. Stop the substrate reaction by washing wells 3 times with 200 µl/well distilled water.
8. Air-dry the plate. Count spots using a dissecting microscope or automated ELISPOT plate reader. Store plates in the dark prior to reading.

## Cytokine ELISPOT Buffers

- ELISA/ELISPOT Coating Buffer Powder
  - Reconstitute powder to 1 L in $dH_20$; filter sterilize using a 0.22 µM filter
- Complete RPMI-1640:
  - RPMI-1640 with 10% FBS and 1% Pen/Strep/L-Glu
- Assay Diluent (supplied as 5X):
  - Dilute 5X solution to 1X in DI $H_2O$
- ELISPOT Wash Buffer:
  - 1X PBS with 0.05% Tween-20 (0.5 ml Tween-20 in 1 L PBS) or eBioscience ELISA Wash Buffer Powder (Cat # 00-0400)
- 1X PBS:
  - 80.0 g NaCl
  - 11.6 g $Na_2HPO_2$
  - 2.0 g $KH_2PO_4$
  - 2.0 g KCl
  - Qs with DI $H_20$ up to 10.0 L, pH to 7.0



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- AEC (3-amino-9-ethyl carbazole) Substrate Solution:
  - AEC Stock Solution: Dissolve 100 mg of AEC in 10 ml of N,N Dimethylformamide (DMF; Pierce, Cat. No. 20672)
  - Add 333 µl of AEC Stock Solution to 10 ml of 0.1 M Acetate Solution (pH 5.0) (see below for recipe). Filter through a 0.45 µm filter.
  - Just before use, add 5 µl of 30% $H_2O_2$. Mix and use immediately.

- 0.1 M Acetate Solution (pH 5.0):
  - Combine 148 ml 0.2 M acetic acid (11.55 ml glacial acetic acid per 1 L $dH_2O$) with 352 ml 0.2 M sodium acetate (27.2 g sodium acetate per 1 L $dH_2O$).
  - Qs to 1 L with $dH_2O$. Adjust pH to 5.0.

## Selected References

1. Gebauer BS, et al. 2002. Evolution of the enzyme-linked immunosorbent spot assay for post-transplant alloreactivity as a potentially useful immune monitoring tool. Am. J. Transplant. 9: 857-866.
2. Guerkov RE, et al. 2003. Detection of low-frequency antigen-specifi c IL-10-producing CD4(+) T cells via ELISPOT in PBMC: cognate vs. nonspecifi c production of the cytokine. J. Immunol. Methods. 279: 111-121.
3. Kreher CR, et al. 2003. CD4+ and CD8+ cells in cryopreserved human PBMC maintain full functionality in cytokine ELISPOT assays. J. Immunol. Methods. 278: 79-93.
4. Ott PA, et al. 2004. CD28 costimulation enhances the sensitivity of the ELISPOT assay for detection of antigen-specifi c memory effector CD4 and CD8 cell populations in human diseases. J. Immunol. Methods. 285: 223-235.
5. Smith JG, et al. 2001. Development and validation of a gamma interferon ELISPOT assay for quantitation of cellular immune responses to varicella-zoster virus. Clin. Diag. Lab. Immunol. 8: 871-879.
6. Shafer-Weaver K, et al. 2003. The Granzyme B ELISPOT assay: an alternative to the 51Cr-release assay for monitoring cell-mediated cytotoxicity. J. Translational. Med. 1: 14.
7. Rininsland F, et al. 2000. Granzyme B ELISPOT assay for ex vivo measurements of T cell immunity. J. Immunol. Meth. 240:143-155.



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