Bovine CCL8 Do-It-Yourself ELISpot

Catalog Number: SPOT2331B-004

Storage: 2-8ºC

Stability: 12 months (from date of receipt)

Country of Origin: USA

Description: Contains Capture Antibody, Standard Control, and Detection Antibody for development of up to two (2) ELISpot assays. Other required materials and reagents are not supplied. The antibodies have been determined to function in an ELISpot with the Standard Control provided. Components may also be purchased separately.

Included Components:

Description	Usage	Quantity	Catalog Number
Anti-Bovine CCL8 Polyclonal Antibody	Capture Antibody	100 µg	KP2033B-100
Recombinant Bovine CCL8	Standard Control	5 µg	RP1903B-005
Biotinylated Anti-Bovine CCL8 Polyclonal Antibody	Detection Antibody	50 µg x 2	KPB2034B-050

Additional Materials and Reagents Required:

Material/Reagent	Suggested Formulation
96-well ELISpot Plate	96-well, sterile, filter membrane ELISpot plate *ELISpot Plate: Catalog # AR2307-001*
Sterile DPBS	0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01M potassium chloride, pH 7.4, sterile filtered
Complete Medium/ Blocking Buffer	Sterile medium containing 10% heat-inactivated Fetal Bovine Serum (FBS) or Equine Serum (ES) in desired cell culture medium, such as RPMI. If required, serum-free medium for cell culture can also be used.
Reagent Diluent	4% BSA in DPBS, 0.2 µm filtered
Wash Buffer	0.05% Tween®-20 in DPBS
Streptavidin-AP	Enzymatic reagent to react with biotinylated detection antibody *Streptavidin-AP: Catalog # AR2308-001*
BCIP/NBT Substrate	5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium substrate to react with AP. *BCIP/NBT Substrate: Catalog # AR2309-025*

Time Requirements:

1 overnight incubation	Coated plate preparation
1-2 overnight incubation	Cell stimulation as desired
2.25+ hours	Assay development

Reagents for Animal Model and Animal Health Research

1000 Westgate Drive, St. Paul, MN 55114
Customer.Service@KingfisherBiotech.com
KingfisherBiotech.com
651.646.0089

Do-It-Yourself ELISpot

Suggested ELISpot Controls:

Control	Suggested Formulation
Standard Control	Recombinant Protein at 50 ng/mL in Complete Medium.
Blank Control	Complete Medium only.
Positive Control	Cells incubated with a polyclonal activator such as PHA, PMA/Ionomycin, ConA, or other, as desired.
Negative Control	Cells in Complete Medium only.

ELISpot Protocol:

Sterile Conditions Suggested:

1. Prepare Capture Antibody Working Solution in DPBS at 4 µg/mL, or as desired.
2. Add 100 µL of Capture Antibody Working Solution to each well.
3. Cover plate and incubate at room temperature (20-25°C) for 12-24 hours.
4. Empty Capture Antibody Working Solution from each well.
5. Add 250 µL of Complete Medium/Blocking Buffer to each well.
6. Cover plate and incubate at room temperature for 1-3 hours.
   Tip: Prepare controls and samples for ELISpot during Blocking Buffer incubation.
7. Empty Blocking Buffer from each well.
8. Add 100 µL of controls or samples to appropriate wells.
   Note: Run each control or sample in triplicate.
9. Cover plate and incubate at 37°C, 5% CO_2 for 12-48 hours, as desired.
   DO NOT DISTURB PLATE DURING INCUBATION!

No Sterile Conditions Required:

10. Wash plate SIX times with Wash Buffer.
    Note: Vigorous plate washing is essential; however, care should be taken to avoid puncturing the filter membrane ELISpot plate during wash steps. If using an automatic plate washer, confirm and adjust the height of the manifold dispenser prior to use. Take care to avoid microbial contamination of equipment. Automated plate washers can easily become contaminated thereby causing assay variability.
11. Prepare Detection Antibody Working Solution in Reagent Diluent at 4 µg/mL, or as desired.
12. Add 100 µL of Detection Antibody Working Solution to each well.
13. Cover plate and incubate at room temperature for 1 hour.
14. Wash plate SIX times with Wash Buffer as described in step 10.
15. Prepare Streptavidin-AP in Reagent Diluent at desired working concentration.
16. Add 100 µL of Streptavidin-AP Working Solution to each well.
17. Cover plate and incubate at room temperature for 1 hour.
18. Wash plate SIX times with Wash Buffer as described in step 10.
19. Add 100 µL of BCIP/NBT Substrate Solution to each well.
20. Develop the plate at room temperature for up to 15 minutes.
21. Stop reaction by flooding the plate with ultrapure water. Remove under tray and flood bottom of tray with ultrapure water.
22. Dry plate and evaluate results.

Warranty:

Reagents for Animal Model and Animal Health Research

1000 Westgate Drive, St. Paul, MN 55114
Customer.Service@KingfisherBiotech.com
KingfisherBiotech.com
651.646.0089