Goose Cluster of Differentiation 8 (CD8) ELISA Kit

Cat.No: MBS010466

Store All Reagents At 2°C-8°C !
Package Size: 48T/Kit or 96T/Kit
Valid Period: Six Months (2°C-8°C)
IN VITRO RESEARCH USE ONLY!
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

1. Introductions

This **Quantitative Sandwich ELISA** kit is only for in vitro research use only, not for drug, household, therapeutic or diagnostic applications! This kit is intended to be used for determination the level of CD8 (hereafter termed this analyte) in **undiluted original Goose body fluids, tissue homogenates, secretions and feces samples**.

2. Performances

Sensitivity: The sensitivity of this kit is 1.0ng/ml.

Detection Range: The detection range of this kit is 3.12ng/ml-100ng/ml.

Specificity: No significant cross-reactivity or interference between this analyte and analogues is observed.

Reproducibility: Both Intra-assay CV (%) and Inter-assay CV (%) is less than 15%. [CV(%) = SD/mean ×100].

3. Materials Supplied

Items	Materials	48 Tests	96 Tests
1	Microelisa Stripplate	12×4 Strips	12×8 Strips
2	Standards×6 vials	0.5ml×6 vials	0.5ml×6 vials
3	Sample Diluent	3.0ml	6.0ml
4	HRP-Conjugate Reagent	5.0ml	10.0ml
5	20×Wash Solution	15ml	25ml
6	Stop Solution	3.0ml	6.0ml
7	Chromogen Solution A	3.0ml	6.0ml
8	Chromogen Solution B	3.0ml	6.0ml
9	Closure Plate Membrane	2	2
10	Sealed Bags	1	1
11	Instruction	1	1

Note: The concentration gradients of Standards from S_{VI} to S_I are followed by: 100,50,25,12.5,6.25,3.12ng/ml.

4. Materials Required but Not Supplied

4.1) Distilled or deionized water.

4.2) Absorbent paper for blotting the plate.

4.3) Precision pipettes and Disposable pipette tips.

4.4) Microplate reader capable of measuring absorbance at 450 nm.

4.5) An incubator which can provide stable incubation conditions up to 37°C±0.5°C.

5. Precautions

5.1) Limited by current skill and knowledge, it is impossible for us to complete the cross-reactivity detection between this analyte and all it's analogues, therefore, cross reaction may still exist in other species or materials.

5.2) Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.

5.3) The reagents and the plate of this kit and it's technical design parameters are only matched and designed for optimal performance for the undiluted original samples in this assay. Owing to the possibility of mismatching between antigen from other resource and antibody used in this kit (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this kit, so please do not substitute reagents from one kit to another and use only the reagents supplied by manufacturer, and moreover, we will not responsibility for using this kit or part of this kit to do any other experiments (such as western blot, immunohistochemistry, spike/recovery and so on) arbitrarily.

5.4) Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.

5.5) Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.

5.6) The Stop Solution suggested for use with this kit is an acid solution, so please pay enough attention to safety when use it. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

6. Samples Collection and Storage

6.1) **Serum** - Allow samples to clot for two hours at room temperature or overnight at 2°C-8°C before centrifugation for approximately 20 minutes at approximately 1000 × g (or 3000 rpm). Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

6.2) **Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for approximately 20 minutes at 1000 × g (or 3000 rpm) at 2°C-8°C within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

6.3) **Blood** - Collect blood using EDTA or heparin as an anticoagulant. Centrifuge samples for approximately 20 minutes at 1000 × g (or 3000 rpm) at 2°C-8°C within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

6.4) **Other Body Fluids (Lymph Fluid and Cerebrospinal Fluid)** - Collect and centrifuge samples for approximately 20 minutes at 1000 × g (or 3000 rpm) at 2°C-8°C within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

6.5) **Tissue Homogenates** - The preparation of tissue homogenates will vary depending upon tissue type. Minced the tissues to small pieces and homogenized them in a certain amount of PBS (Usually 10mg tissue to 100μl PBS.). After that, centrifugate homogenates for approximately 15 minutes at 1500×g (or 5000 rpm). Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

6.6) **Secretions (Synovial Fluid, Saliva, Urine and so on)** - Collect and centrifuge samples for approximately 20 minutes at 1000 × g (or 3000 rpm) at 2°C-8°C within 30 minutes after collection. Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

6.7) **Feces** - Collect and fully shaking samples in a certain amount of PBS (Usually 10mg tissue to 100μl PBS.). After that, centrifuge samples for approximately 15 minutes at 1500×g (or 5000 rpm). Collect the supernatants carefully, assay immediately or store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Important Notes:

6.8.1) We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please make sure that sufficient samples are available.

6.8.2) Fresh samples without long time storage are recommended for assay. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C(≤one month) or -80°C(≤two months) to avoid loss of bioactivity and contamination. Avoid repeated freeze/thaw cycles.

6.8.3) Grossly hemolyzed samples are not suitable for use in this assay, so the samples should be centrifugated adequately and no hemolysis or granule was allowed.

6.8.4) The kit can not assay the samples which contain sodium azide(NaN3), because NaN3 will inhibit the activity of horseradish peroxidase (HRP).

6.8.5) If the samples are not indicated in the manual, a preliminary experiment to determine the validity of this kit is necessary.

7. Reagent Preparation and Storage

Please store the plate and all reagents at 2°C-8°C!

7.1) The valid period of this kit is six months at 2°C-8°C. The kit should not be used beyond the expiration date.

7.2) Wash Solution (1×) - Dilute one volume of Wash Solution (20×) with nineteen volumes of deionized or distilled water. Diluted Wash Solution is stable for one month at 2°C-8°C. Undiluted Wash Solution and other reagents are stable for six months at 2°C-8°C.

7.3) When the kit is opened, please used up all Microelisa Stripplate as soon as possible after removed the plate from the foil pouch. The Microelisa Stripplate is detachable, so please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal for preventing damps. The remaining reagents still need to be stored at 2°C-8°C.

8. Assay Procedures

Please do the experiments strictly to follow the assay procedures and DO NOT change any assay procedures arbitrarily!

8.1) Bring all reagents and samples to room temperature (18°C-25°C) naturally for 30min before starting assay procedures, you can do a low - speed centrifugation for one or two seconds to concentrate the Standards to the bottom of the vials if necessary. **DO NOT** use hot water baths to thaw samples or reagents. It is recommended that all Standards and samples be added in duplicate to the plate.

8.2) Set Standard wells, Sample wells and Blank/Control wells, add Standard 50µl to each Standard well, add Sample 50µl to each Sample well, add Sample Diluent 50µl to each Blank/Control well.

8.3) Add 100µl of HRP-conjugate reagent to **each well**, cover with an adhesive strip and incubate for 60 minutes at 37°C.

8.4) Wash the Microtiter Plate 4 times.

8.4.1) **Manual Washing** - Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Solution (1×), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. **Note**: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

8.4.2) **Automated Washing** - Aspirate all wells, then wash plates four times using Wash Buffer (1×). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µl/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

8.5) Add Chromogen Solution A 50µl and Chromogen Solution B 50µl to **each well** successively. Gently mix and then **protect from light** to incubate for 15 minutes at 37°C.

8.6) Add 50µl Stop Solution to **each well**. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8.7) Read the Optical Density (O.D.) at 450 nm using a Microelisa Stripplate reader within 15 minutes after adding Stop Solution.

8.8) **Important Notes:**:

8.8.1) Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.

8.8.2) Do not remove the plate from the foil pouch until needed. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results.

8.8.3) The concentration gradients of Standards of this kit have already covered far more than the range of concentration of this analyte in undiluted original samples, so please **DO NOT** use the **diluted** or **non-original** samples when using our kits, please assay the undiluted original samples directly, otherwise samples that prepared by chemical lysis buffer may cause unexpected ELISA results or contaminated, and moreover, the level of this analyte that has been diluted may out of the detection range of this kit.

8.8.4) The Sample Diluent are more than PBS, it also contains a little stabilizer and preservative. It is made well as a Blank/Control reagent(adjusted zero value) for the experiment, because the Standards also contain a little stabilizer and preservative, so **DO NOT** use your own PBS or other reagents as a Blank/Control reagent, even if you have used it to collect your samples.

8.8.5) Samples or Reagents Addition: Please carefully add samples to wells and mix gently to avoid foaming. **DO NOT** touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and samples, although not required, is recommended. To avoid contamination, please use fresh disposable pipette tips for each transfer.

8.8.6) Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. **DO NOT** allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, **DO NOT** let the strips **DRY** at any time during the assay. Incubation time and temperature must be observed.

8.8.7) Washing Plate: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading.

8.8.8) Controlling of Reaction Time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. The color developed in the wells will turn from blue to yellow after added the Stop Solution. If the color turns green, it indicate the Stop Solution has not mixed thoroughly.

8.8.9) Chromogen Solution B is easily contaminated, it should remain colorless or light blue until added to the plate, please protect it from light.

9. Calculation of Results

9.1) Average the duplicate readings for each standard and sample to subtract average optical density of the Blank/Control ($V_{B/C}$).

Standards (concentration):	0	S_I	S_{II}	S_{III}	S_{IV}	S_V	S_{VI}
Mean O.D.(450nm):	$V_{B/C}$	V_1	V_2	V_3	V_4	V_5	V_6
Adjusted:	0	V_1-$V_{B/C}$	V_2-$V_{B/C}$	V_3-$V_{B/C}$	V_4-$V_{B/C}$	V_5-$V_{B/C}$	V_6-$V_{B/C}$

9.2) Using the professional curve fitting software to make a standard curve (usually most of the curves are linear, and a few curves are quadratic or cubic) and calculate the level of this analyte.

9.3) **Note:** Any variation in operation, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain his own standard curve.