Mouse IL-4 Precoated ELISPOT Kit

Cat#:2210402

[Product Description]

This kit is a ready-to-use kit, including Detection Antibody, Streptavidin-HRP, AEC Coloring System, Dilution Buffer, Cell Stimulant, Washing Buffer, Pre-coating PVDF Plate, which can meet the needs of ELISPOT experiments.This kit is only used for scientific research. It shall not be used for diagnosis. Read the instruction and check the components of the kit before use. If there are any questions, please contact with Shenzhen Dakewe Bio-engineering Co., Ltd.

[Materials Provided]

Product Name	Cat No.	Component Name	Component Size	Quantity
Mouse IL-4 Precoated ELISPOT Kit	2210402	Biotinylated Antibody	100 μL	1 vial
		Streptavidin-HRP	100 μL	1 vial
		Cell Stimulant	50 T	1 bottle
		Dilution Buffer R(1×)	10 mL	2 bottles
		Washing Buffer (50×)	15 mL	1 bottle
		AEC Dilution	10 mL	1 bottle
		AEC Solution I (20×)	500 μL	1vial
		AEC Solution II (20×)	500 μL	1vial
		AEC Solution III (200×)	50 μL	1vial
		Pre-coated PVDF Plate	/	1 plate

[Self-provided Items]

1. Cell culture medium (It is recommended to use serum-free medium or RPMI-1640 with 100 units/mL Penicillin,100 μg/mL Streptomycin and 5%~10% fetal calf serum)
2. Lymphocyte Separation Medium
3. Clean bench
4. CO_2-incubator
5. Multi-channel Pipette, pipette tips
6. A microscope or an immune spot image analyzer for spot counting

[Preparation of Reagents]

1. **Washing Buffer (50×):** Diluted with redistilled water to prepare 1×Washing Buffer at the ratio of 1:50 for standby.
2. **Cell Stimulant:** Sterile PBS is added to resuspend lyophilized powder according to the label information on the vial.
3. **Biotinylated Antibody:** Diluted with Dilution Buffer R (1×) (1:100).
4. **Streptavidin-HRP:** Diluted with Dilution Buffer R (1×) (1:100).
5. **AEC Coloring System:** By referring to the following table, AEC Dilution, AEC Solution I (20×), AEC Solution II (20×), AEC Solution III (200×) are mixed well in a clean container at the ratio of 180:10:10:1. The half-life of the AEC coloring solution is about 30 minutes, and the solution is prepared prior to use.

Total volume	AEC Dilution	AEC Solution I(20×)	AEC Solution II(20×)	AEC Solution III(200×)
1 mL	0.9 mL	50 μL	50 μL	5 μL
2 mL	1.8 mL	100 μL	100 μL	10 μL
3 mL	2.7 mL	150 μL	150 μL	15 μL
4 mL	3.6 mL	200 μL	200 μL	20 μL
5 mL	4.5 mL	250 μL	250 μL	25 μL
8 mL	7.2 mL	400 μL	400 μL	40 μL
10 mL	9 mL	500 μL	500 μL	50 μL

[Operation Process]

Day 1: Cell activation (aseptic operation is required)

1. Pre-coating plate: The PVDF membrane is wetted by adding 200 μL of culture medium to the pre-coating plate, and then it shall be pulled out after standing at room temperature for 5-10 minutes.
2. The 100 μL of cell suspension is added to each well. And the cell concentration is adjusted according to different mitogen or antigen. Only 100 μL of medium is added to the negative control well.
3. The 10 μL of appropriate concentration of antigen is added (e.g. cell stimulant) to each well.
4. Incubation: After all the samples and stimulants are added, the plate is covered with lid. And the incubation is performed at an incubator with 37°C, 5% CO_2 for 16~24 hours. The specific incubation time varies due to different cell types and cytokines.

Day 2: Operation after incubation (aseptic operation is no longer required)

5. The cells and medium in the wells are shaken off; 200 μL of ice-cold deionized water is added to the wells, and placed in a refrigerator at 2°C~8°C for 10 minutes to remove the cells through low permeability and pyrolysis.
6. The liquid in the well is shaken off; 260 μL of 1×Washing Buffer is added to each well. The well is washed 6 times, each time for 60 seconds. The plate strips are dried by turning it over and tapping it on the absorbent paper.
7. The 100 μL of diluted Biotinylated Antibody is added to each well; the plate is covered with lid, and the incubation is performed at 37°C for 1 hour.
8. Repeat Step 6.
9. The 100 μL of diluted Streptavidin-HRP is added to each well; the plate is covered with lid, and the incubation is performed at 37°C for 1 hour.
10. Repeat Step 6.
11. The 100 μLof freshly prepared AEC coloring solution is added to each well. Stand at a dark place and at room temperature for 5~30 minutes. Note: The spot shape is checked every 5~10 minutes until the spot is clear.
12. The liquid in the well is poured; the base of the plate is uncovered, and the front and back of each experimental well and the base are washed with a large amount of distilled water. Excess water is shaken off; the plate is placed in a cool place and at room temperature. Afterwards, the base is installed after the plate is naturally dried.
13. The spots are counted manually under microscope or by automatic ELISPOT analyzer.

[Warnings & Precautions]

Warnings

1. This kit is only used for scientific researches. It shall not be used for diagnosis and treatment.
2. Do not use kits that are beyond the expiration date or with damaged packaging.
3. Do not use this kit in conjunction with the components of other kits.
4. Wear gloves and avoid direct contact with the skin, when using chemical reagents or biological products, especially using AEC substrates containing dimethylformamide (DMF) which is harmful to the skin and eyes. Once they are stuck to your skin, please immediately rinse with plenty of water and seek medical attention in time.
5. Please dispose of unused reagents or biological products according to local laws and regulations.
6. Note: Pipetting, washing the plate, incubation time and temperature, cell conditions and other factors are related to the formation of spots, the degree of color development and the changes in the background.

Precautions

1. The AEC substrate is prepared on the spot, mixed upside down, and used within half an hour; Avoid containers made of polystyrene (PS); If the reagent precipitates during normal use, the reagent is mixed at intervals during the sample adding process, and slight precipitation does not affect the experimental results.
2. Do not touch the membrane directly with pipette tips, so as not to scratch or damage the membrane.
3. After completion of the experiment, do not dry the plate at temperature higher than 37ºC, otherwise the membrane will be broken.
4. Before terminating the reaction, lift the base baffle and flush the base liquid to prevent the membrane background from aggravating, but please operate gently to avoid direct damage to the membrane.

[Storage]

The kit is stored at 2ºC~8ºC and can be stored stably for 12 months. The kit cannot be used after expiration, and remains sterile after opening.

[Company Information]

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