

ELISA Buffer Set (Phosphate system,96T)

Catalog Number: EBS-002

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96T)	Format	Storage	
				Unopened	Opened
EBS002-C01	High-bind plate	1 plate	Powder	2-8°C	2-8°C
EBS002-C02	Coating buffer	12 mL	Liquid	2-8°C	2-8°C
EBS002-C03	10xWashing Buffer	60 mL	Liquid	2-8°C	2-8°C
EBS002-C04	Blocking buffer	50 mL	Liquid	2-8°C	2-8°C
EBS002-C05	Substrate solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
EBS002-C06	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

SRORAGE

1. The unopened kit is stable for 12 months from the date of manufacture if stored at 2°C to 8°C.
2. The opened kit should be stored per Table 1. The shelf life is 30 days from the date of opening.

Note: a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

c. The reagent should be properly balanced to room temperature before use.

APPLICATION

1. High-bind plate (EBS002-C01): 96 well microplates. Used for solid phase adsorption of samples in coated solution.
2. Coating buffer (EBS002-C02): Coated buffer for diluting coated sample.
3. 10xWashing Buffer (EBS002-C03): For washing the enzyme label plate, 10xWashing Buffer should be diluted with purified water to 1xWashing Buffer before use (For example, 60 mL 10xWashing Buffer+ 540 mL purified water).
4. Blocking buffer (EBS002-C04): Used to block the enzyme label plate. Diluting the Blocking buffer 4 times with 1xWashing Buffer can be used as a Sample buffer diluent (For example, 1 mL Blocking buffer + 3 mL 1xWashing Buffer).

5. Substrate solution (EBS002-C05): For color reaction.
6. Stop Solution (EBS002-C06): Used to terminate the experiment.

TYPICAL DATA

1. Coating

Dilute Human TNF- α Capture Antibody stock solution (ACROBiosystems Cat.No.CRS-D002, CRD002-C01) to 1.0 $\mu\text{g}/\text{mL}$ with Coating Buffer (EBS002-C02) to make Human TNF- α Capture Antibody working solution.

Description 100 μL of Human TNF- α Capture Antibody working liquid (1.0 $\mu\text{g}/\text{mL}$) is added to each well onto a High-bind plate (EBS002-C01), sealed with a microporous plate film, and incubated at 4°C overnight (or for 16 hours).

2. Washing

Remove the remaining solution by aspiration, add 300 μL of 1 \times Washing Buffer to each well, gently tap the plate for 1 minute, remove any remaining 1 \times Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

3. Blocking

Add 300 μL Blocking Buffer (EBS002-C04) to each well, seal the plate with microplate sealing film and incubate at room temperature for 2.0 hours.

4. Washing

Repeat step 2.

5. Add Samples

The reconstructed Human TNF- α Standard (ACROBiosystems Cat.No.CRS-D002, CRD002-C02) was diluted with Sample buffer, and the dilution range was 4.89-312.5 pg/mL . Add 100 μL Samples to each well. For blank Control wells, please add 100 μL Sample buffer.

6. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

7. Washing

Repeat step 2.

8. Add Human TNF- α Detection Antibody

Dilute Biotinylated-Human TNF- α Detection Antibody (ACROBiosystems Cat.No.CRS-D002, CRD002-C03) stock solution to 0.5 $\mu\text{g}/\text{mL}$ with Sample buffer to make Biotinylated-Human TNF- α Detection Antibody working solution.

For all wells, add 100 μL Biotinylated-Human TNF- α Detection Antibody (0.5 $\mu\text{g}/\text{mL}$) working solution. Please prepare it for one-time use only.

9. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

10. Washing

Repeat step 2.

11. Add Streptavidin-HRP

For all wells, add 100 μL Streptavidin-HRP (ACROBiosystems Cat.No.CRS-D002, CRD002-C04) (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

12. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 30 min.

13. Washing

Repeat step 2.

14. Substrate Reaction

Add 100 μL Substrate Solution (EBS002-C05) to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

15. Termination

Add 50 μL Stop Solution (EBS002-C06) to each well, and tap the plate gently to allow thorough mixing.

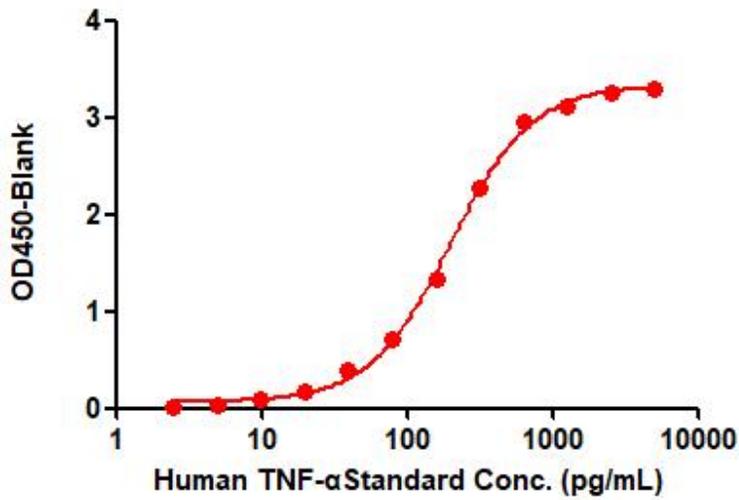
Note: The color in the wells should change from blue to yellow.

16. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note: To reduce the background noise, subtract the value read at OD450nm with the value read at OD630 nm.

17. Data Analysis



Immobilized Human TNF- α Capture Antibody (Cat.No.CRS-D002, CRD002-C01) at 1 $\mu\text{g/mL}$ (100 $\mu\text{L/well}$) can bind Human TNF- α Standard, and then add Biotinylated-Human TNF- α Detection Antibody (Cat.No.CRS-D002, CRD002-C03) at 0.5 $\mu\text{g/mL}$ (100 $\mu\text{L/well}$). Detection was performed using HRP-conjugated streptavidin with a linear range of 4.89-312.5 pg/mL .