

# Zebrafish TNF- $\alpha$ Do-It-Yourself ELISA



**Catalog Number:** DIY1542Z-003  
**Storage:** 2-8°C  
**Stability:** 12 months (from date of receipt)  
**Country of Origin:** USA  
**Alias:** TNFSF2

**Description:** Contains capture antibody, standard, and detection antibody for development of an ELISA. The antibodies have been determined to function in an ELISA with the standard provided. Optimal buffers, concentrations, incubation times, incubation temperatures, and methods for the ELISA have not been determined. **A working knowledge of ELISA is strongly recommended.** The quantities of components provided are not matched. Components may also be purchased separately.

| Included Components: | Description                                                   | Usage              | Quantity    | Catalog Number |
|----------------------|---------------------------------------------------------------|--------------------|-------------|----------------|
|                      | Anti-Zebrafish TNF- $\alpha$ Polyclonal Antibody              | Capture Antibody   | 100 $\mu$ g | KP1540Z-100    |
|                      | Recombinant Zebrafish TNF- $\alpha$                           | Standard           | 5 $\mu$ g   | RP1318Z-005    |
|                      | Biotinylated Anti-Zebrafish TNF- $\alpha$ Polyclonal Antibody | Detection Antibody | 50 $\mu$ g  | KPB1541Z-050   |

| Suggested Reagents: | Reagent                             | Suggested Formulation                                                                                                                                                                                                                                                                                                                                                                                      |
|---------------------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                     | DPBS                                | 0.008M sodium phosphate, 0.002M potassium phosphate, 0.14M sodium chloride, 0.01M potassium chloride, pH 7.4                                                                                                                                                                                                                                                                                               |
|                     | 96-well ELISA Plate                 | Clear, flat-bottom, high-binding 96-well plate, 8-wells per strip, 350 $\mu$ L per well<br><i>ELISA Plates: Catalog # AR0658-005</i>                                                                                                                                                                                                                                                                       |
|                     | Standard and Sample Diluent         | The optimal Standard and Sample Diluent will need to be determined for each sample type to obtain optimal recovery and linearity. The appropriate Standard and Sample Diluent will mimic the sample's response to a known quantity of protein standard and will provide linear results when diluted. Often a 1:4 dilution of the sample in Reagent Diluent will provide acceptable recovery and linearity. |
|                     | Reagent Diluent and Blocking Buffer | 4% BSA in DPBS, 0.2 $\mu$ m filtered                                                                                                                                                                                                                                                                                                                                                                       |
|                     | Wash Buffer                         | 0.05% Tween®-20 in DPBS                                                                                                                                                                                                                                                                                                                                                                                    |
|                     | Streptavidin-HRP                    | Enzymatic reagent to react with biotinylated detection antibody<br><i>Streptavidin-HRP: Catalog # AR0068-001</i>                                                                                                                                                                                                                                                                                           |
|                     | Substrate                           | 3,3',5,5'-tetramethylbenzidine (TMB) Substrate<br><i>ELISA Accessory Pack: Catalog # AR0133-002</i>                                                                                                                                                                                                                                                                                                        |
|                     | Stop Solution                       | 0.18 M Sulfuric Acid<br><i>ELISA Accessory Pack: Catalog # AR0133-002</i>                                                                                                                                                                                                                                                                                                                                  |
|                     | Plate Sealer                        | Adhesive film to prevent evaporation <i>Catalog # AR1962-100</i>                                                                                                                                                                                                                                                                                                                                           |

**Warranty:** Products are warranted by Kingfisher Biotech, Inc. to meet stated product specifications and to conform to label descriptions when used, handled and stored according to instructions. Unless otherwise stated, this warranty is limited to one year from date of sale or expiry date, whichever comes first. Kingfisher Biotech's sole liability for the product is limited to replacement of the product or refund of the purchase price. Kingfisher Biotech products are supplied for research applications. They are not intended for medicinal, diagnostic or therapeutic use. The products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Kingfisher Biotech, Inc.

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## Reagents for Animal Model and Animal Health Research

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# Do-It-Yourself ELISA

**Generic ELISA Protocol:** Optimal buffers, concentrations, incubation times, incubation temperatures, and methods have not been determined. A working knowledge of ELISA is strongly recommended.

1. Prepare Capture Antibody in DPBS at desired working concentration.
2. Add 100  $\mu$ L of Capture Antibody Working Solution to appropriate wells.
3. Cover plate with Plate Sealer and incubate at room temperature (20-25°C) for 12-24 hours.
4. Empty Capture Antibody Working Solution from plate. Blot plate onto paper towels or other absorbent material.
5. Add 250  $\mu$ L of Blocking Buffer to appropriate wells.
6. Cover plate with Plate Sealer and incubate at room temperature for 1-3 hours.
7. Empty Blocking Buffer from plate. Blot plate onto paper towels or other absorbent material.
8. Prepare Standard and sample as desired with Standard and Sample Diluent.
9. Add 100  $\mu$ L of Standard or sample to appropriate wells.  
Note: Run each Standard or sample in duplicate.
10. Cover plate with Plate Sealer and incubate at room temperature for 1 hour.
11. Wash plate FOUR times with Wash Buffer.  
Note: Gently squeeze the long sides of plate frame before washing to ensure all strips remain securely in the frame. Empty plate contents. Use a squirt wash bottle to vigorously fill each well completely with 1X Wash Buffer, then empty plate contents. Repeat procedure three additional times for a total of FOUR washes. Blot plate onto paper towels or other absorbent material.
12. Prepare Detection Antibody in Reagent Diluent at desired working concentration.
13. Add 100  $\mu$ L of Detection Antibody Working Solution to each well.
14. Cover plate with Plate Sealer and incubate at room temperature for 1 hour.
15. Wash plate FOUR times with Wash Buffer as described in step 11.
16. Prepare Streptavidin-HRP in Reagent Diluent at desired working concentration.
17. Add 100  $\mu$ L of Streptavidin-HRP Working Solution to each well.
18. Cover plate with Plate Sealer and incubate at room temperature for 30 minutes.
19. Wash plate FOUR times with Wash Buffer as described in step 11.
20. Add 100  $\mu$ L of TMB Substrate Solution to each well.
21. Develop the plate in the dark at room temperature for 30 minutes or as desired.  
Note: Do **NOT** cover plate with Plate Sealer.
22. Stop reaction by adding 100  $\mu$ L of Stop Solution to each well.
23. Measure absorbance on a plate reader at 450 nm.



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