Fish Tumor necrosis factorα (TNF-α) ELISA Kit

- **For research use only.**
- **For the quantitative in vitro determination of Tumor necrosis factorα (TNF-α)concentrations in Fish serum, plasma, culture media or any biological fluid.**
- **Expiration date：six months .**
- **Storage： 2-8℃.**

Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to TNF-α. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)- conjugated antibody specific for TNF-α is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain TNF-α and HRP conjugated TNF-α antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of TNF-α. You can calculate the concentration of TNF-α in the samples by comparing the OD of the samples to the standard curve.

Materials provided with the kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microelisa stripplate	1	2-8℃
5	Standard：360 pg/ml	0.5ml×1 bottle	2-8℃
6	Standard diluent	1.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃
8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen Solution A	6ml×1 bottle	2-8℃
10	Chromogen Solution B	6ml×1 bottle	2-8℃
11	Stop Solution	6ml×1 bottle	2-8℃
12	wash solution	20ml (30X)×1bottle	2-8℃

Sample preparation

1. **Serum preparation**

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

2. **Plasma preparation**

Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

3. **Urine samples**

Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20

min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

4. **Cell samples**

If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

5. **Tissue samples**

Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4℃. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Notes:

1. Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20 ℃ .Repeated freeze-thaw cycles should be avoided.
2. Our kits can not be used for samples with NaN3 which can inhibit the activity of HRP.

Procedure

1. Dilution of Standards

   Ten wells are set for standards in a Microelisa stripplate. In Well 1 and Well 2, 100µl Standard solution and 50µl Standard Dilution buffer are added and mixed well. In Well 3 and Well 4, 100µl solution from Well 1 and Well 2 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50µl solution from Well 3 and Well 4 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. In Well 7 and Well 8, 50µl solution from Well 5 and Well 6 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50µl solution from Well 7 and Well 8 are added respectively. Then 50µl Standard Dilution buffer are added and mixed well. 50µl solution is discarded from Well 9 and Well 10. After dilution, the total volume in all the wells are 50µl and the concentrations are240 pg/ml,160 pg/ml, 80 pg/ml, 40 pg/ml and 20pg/ml, respectively.

360 pg/ml 240 pg/ml 160 pg/ml 80pg/ml 40pg/ml 20 pg/ml

2. In the Microelisa stripplate, leave a well empty as blank control. In sample wells, 40µl Sample dilution buffer and 10µl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.
4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).
5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
6. Add 50 µl HRP-Conjugate reagent to each well except the blank control well.
7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Notes:

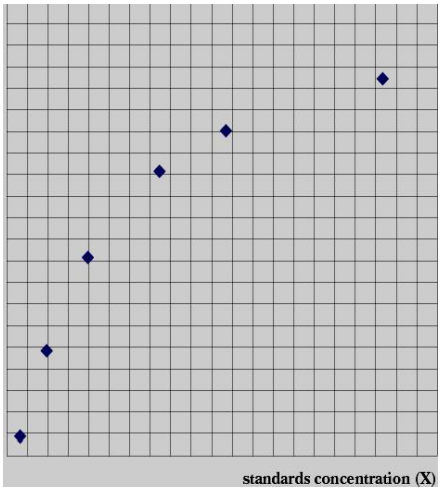
1. Store the kit at 4° C upon receipt.The kit should be equilibrat4ed to room temperature before the assay. Remove any unneeded strips from Fish TNF-α Antibody-Coated plate, reseal them in zip-lock foil and keep at 4° C.
2. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
3. Accurate pipette should be used to avoid experimental error. Samples should be added to the Microplate in less than 5 minutes. If a large number of samples are included, multiple channel pipette is recommended.
4. Standard curve should be included in every assay. Replicate wells are recommended. If the OD value of the sample is greater than the first well of standards, please dilute the sample (n times) before test. When calculating the original TNF-α concentration, please

multiply the total dilution factor (XnX5).

5. In order to avoid cross-contamination, Microplate sealers are for one-time use only.
6. Please keep Substrate away from light.
7. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microtiter Plate Reader.
8. All the samples, washing buffer and wastes should be treated as infectious agents.
9. Reagents from different lots should not be mixed.

Calculation of Results

Known concentrations of Fish TNF-α Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Fish TNF-α in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.



This diagram is for reference only

Kit performance

1. Correlation coefficient (R) of linear regression of the samples is more than 0.92
2. The difference in intra-assay and inter-assay is less than 9% and 15% respectively.

Assay range

4pg/ml-300 pg/ml

鱼肿瘤坏死因子α（TNF-α）酶联免疫分析(ELISA)试剂盒使用说明书

- 本试剂盒仅供科研使用。
- 本试剂盒用于体外定量检测鱼血清、血浆、组织、细胞上清及相关液体样本中肿瘤坏死因子α（TNF-α）的含量。
- 有效期：6个月
- 保存条件：2-8℃

实验原理

本试剂盒应用双抗体夹心法测定标本中鱼肿瘤坏死因子α（TNF-α）水平。用纯化的鱼肿瘤坏死因子α（TNF-α）抗体包被微孔板，制成固相抗体，往包被单抗的微孔中依次加入肿瘤坏死因子α（TNF-α），再与HRP标记的肿瘤坏死因子α（TNF-α）抗体结合，形成抗体-抗原-酶标抗体复合物，经过彻底洗涤后加底物TMB显色。TMB在HRP酶的催化下转化成蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅和样品中的肿瘤坏死因子α（TNF-α）呈正相关。用酶标仪在450nm波长下测定吸光度（OD值），通过标准曲线计算样品中鱼肿瘤坏死因子α（TNF-α）浓度。

试剂盒组成

	试剂盒组成	96 孔配置	保存
1	说明书	1 份	
2	封板膜	2 片（96）	
3	密封袋	1 个	
4	酶标包被板	1×96	2-8℃保存
5	标准品：360pg/ml	0.5ml×1 瓶	2-8℃保存
6	标准品稀释液	1.5ml×1 瓶	2-8℃保存
7	酶标试剂	6 ml×1 瓶	2-8℃保存
8	样品稀释液	6 ml×1 瓶	2-8℃保存
9	显色剂 A 液	6 ml×1 瓶	2-8℃保存
10	显色剂 B 液	6 ml×1 瓶	2-8℃保存

11	终止液	6ml×1 瓶	2-8℃保存
12	浓缩洗涤液	（20ml×30 倍）×1 瓶	2-8℃保存

样本处理及要求

1. 血清：室温血液自然凝固 10-20 分钟，离心 20 分钟左右（2000-3000 转/分）。仔细收集上清，保存过程中如出现沉淀，应再次离心。
2. 血浆：应根据标本的要求选择 EDTA 或柠檬酸钠作为抗凝剂，混合 10-20 分钟后，离心 20 分钟左右（2000-3000 转/分）。仔细收集上清，保存过程中如有沉淀形成，应该再次离心。
3. 尿液：用无菌管收集，离心 20 分钟左右（2000-3000 转/分）。仔细收集上清，保存过程中如有沉淀形成，应再次离心。胸腹水、脑脊液参照实行。
4. 细胞培养上清：检测分泌性的成份时，用无菌管收集。离心 20 分钟左右（2000-3000 转/分）。仔细收集上清。检测细胞内的成份时，用 PBS（PH7.2-7.4）稀释细胞悬液，细胞浓度达到 100 万/ml 左右。通过反复冻融，以使细胞破坏并放出细胞内成份。离心 20 分钟左右（2000-3000 转/分）。仔细收集上清。保存过程中如有沉淀形成，应再次离心。
5. 组织标本：切割标本后，称取重量。加入一定量的 PBS，PH7.4。用液氮迅速冷冻保存备用。标本融化后仍然保持 2-8℃的温度。加入一定量的 PBS（PH7.4），用手工或匀浆器将标本匀浆充分。离心 20 分钟左右（2000-3000 转/分）。仔细收集上清。分装后一份待检测，其余冷冻备用。
6. 标本采集后尽早进行提取，提取按相关文献进行，提取后应尽快进行实验。若不能马上进行试验，可将标本放于-20℃保存，但应避免反复冻融.
7. 不能检测含 NaN3 的样品，因 NaN3 抑制辣根过氧化物酶的（HRP）活性。

操作步骤

1. 标准品的稀释与加样：在酶标包被板上设标准品孔 10 孔，在第一、第二孔中分别加标准品 100μl，然后在第一、第二孔中加标准品稀释液 50μl，混匀；然后从第一孔、第二孔中各取 100μl 分别加到第三孔和第四孔，再在第三、第四孔分别加标准品稀释液 50μl，混匀；然后在第三孔和第四孔中先各取 50μl 弃掉，再各取 50μl 分别加到第五、第六孔中，再在第五、第六孔中分别加标准品稀释液 50ul，混匀；混匀后从第五、第六孔中各取 50μl 分别加到第七、第八孔中，再在第七、第八孔中分别加标准品稀释液 50μl，混匀后从第七、第八孔中分别取 50μl 加到第九、第十孔中，再在第九第十孔分别加标准品稀释液 50μl，混匀后从第九第十孔中各取 50μl 弃掉。（稀释后各孔加样量都为 50μl，浓度分别为 240pg/ml，160pg/ml，80 pg/ml，40pg/ml， 20 pg/ml）。

360 pg/ml　240 pg/ml　160 pg/ml　80 pg/ml　40 pg/ml　20 pg/ml

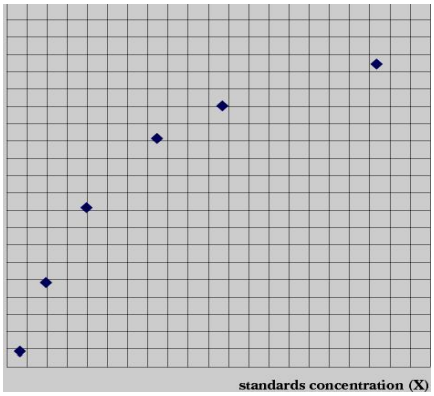
2. 加样：分别设空白孔（空白对照孔不加样品及酶标试剂，其余各步操作相同）、待测样品孔。在酶标包被板上待测样品孔中先加样品稀释液 40μl，然后再加待测样品 10μl（样品最终稀释度为 5 倍）。加样将样品加于酶标板孔底部，尽量不触及孔壁，轻轻晃动混匀。
3. 温育：用封板膜封板后置 37℃温育 30 分钟。
4. 配液：将 30 倍浓缩洗涤液用蒸馏水 30 倍稀释后备用。
5. 洗涤：小心揭掉封板膜，弃去液体，甩干，每孔加满洗涤液，静置 30 秒后弃去，如此重复 5 次，拍干。
6. 加酶：每孔加入酶标试剂 50ul，空白孔除外。
7. 温育：操作同 3。
8. 洗涤：操作同 5。
9. 显色：每孔先加入显色剂 A50ul，再加入显色剂 B50ul，轻轻震荡混匀，37℃避光显色 15 分钟.
10. 终止：每孔加终止液 50ul，终止反应（此时蓝色立转黄色）。
11. 测定：以空白孔调零，450nm 波长依序测量各孔的吸光度（OD 值）。 测定应在加终止液后 15 分钟以内进行。

注意事项

1.试剂盒从冷藏环境中取出应在室温平衡 15-30 分钟后方可使用，酶标包被板开封后如未用完，板条应装入密封袋中保存。
2.浓洗涤液可能会有结晶析出，稀释时可在水浴中加温助溶，洗涤时不影响结果。
3.各步加样均应使用加样器，并经常校对其准确性，以避免试验误差。一次加样时间最好控制在 5 分钟内，如标本数量多，推荐使用排枪加样。
4.请每次测定的同时做标准曲线，最好做复孔。如标本中待测物质含量过高（样本 OD 值大于标准品孔第一孔的 OD 值），请先用样品稀释液稀释一定倍数（n 倍）后再测定，计算时请最后乘以总稀释倍数（×n×5）。
5.封板膜只限一次性使用，以避免交叉污染。
6.底物请避光保存。
7.严格按照说明书的操作进行，试验结果判定必须以酶标仪读数为准.
8.所有样品，洗涤液和各种废弃物都应按传染物处理。
9.本试剂不同批号组分不得混用。
10. 如与英文说明书有异，以英文说明书为准。

计算

以标准物的浓度为横坐标，OD 值为纵坐标，在坐标纸上绘出标准曲线，根据样品的 OD 值由标准曲线查出相应的浓度；再乘以稀释倍数；或用标准物的浓度与 OD 值计算出标准曲线的直线回归方程式，将样品的 OD 值代入方程式，计算出样品浓度，再乘以稀释倍数，即为样品的实际浓度。



（此图仅供参考）

试剂盒性能

1.样品线性回归与预期浓度相关系数 R 值为 0.92 以上。
2.批内与批间应分别小于 9%和 15%

检测范围：

4 pg/ml -300 pg/ml