Human Interferon α(IFN-α)ELISA Kit Instruction

Applicable for the tests of following sample (applicable sample with black background)

Serum, blood plasma **Tissue homogenate** **Ascites** **Cell culture supernatants**

MBS006588

Only for research purpose, not for clinical diagnose.

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1.Experiment principle: This test kit applies competition method to detect the content of Human Interferon α(IFN-α). Add samples to enzyme well which has been pre-coated with antibodies, then add recognition antigen labeled by horse radish peroxidase (HRP); after been incubated 1 hour at 37 ℃, both compete with solid phase antigen and formed immune complex; after been washing by PBST, the combined HRP catalyses TMB (Tetramethyl benzidine) into blue, and turns into yellow by the action of acid; it has absorption peak under 450nm wavelength, and its absorbance is negatively correlated with antigen density of sample.

1. Configuration:

Configuration	48 wells	96 wells	Preservation
Instruction	1	1	
Closure plate membrane	2 sheet (48)	2 sheet (96)	
Sealing bags	1	1	
Precoated plate	1×48	1×96	2-8℃
Standard:2400ng/L	0.5ml×1	0.5ml×1	2-8℃
Standard/Sample diluent	3ml×1	6ml×1	2-8℃
enzyme-labeled reagent	3ml×1	6ml×1	2-8℃
Chromogen Solution A	3ml×1	6ml×1	2-8℃
Chromogen Solution B	3ml×1	6ml×1	2-8℃
Stop Solution	3ml×1	6ml×1	2-8℃
Washing Solution	(20ml×25)×1	(20ml×25)×1	2-8℃

Note:

a.“Sample diluent” is PBS of 0.05M; “Stop solution” is H2SO4 of 2M; and “Washing concentrate” is PBST with0.15% of tween-20 which could be self-prepared if it is not sufficient.

b.Different company has different buffer system. Please do not mix with reagent of other companies so as not to affect results.

c.The washing concentrate will dissolve out few salt under low temperature, and it will disappear by heating without any influence.

2. Preservation

It can be preserved 6 months under temperature of 2-8℃, and longer under temperature of -20 ℃. The enzyme-labeled plate is vacuum-packed, and its strip can be dismounted; if there is any left after first unpacked, please put the remaining into supplied sealing bags together with desiccant; it is valid within one month if preserved under temperature of 2-8℃. The reagent shall not be frozen-thawed repeatedly.

3. Reagent and tools required but not supplied

1.）Incubator of 37℃

2.）Standard Enzyme reader

3.）Precision pipettes and Disposable pipette tips

4.）Double-distilled water or ultrapure water

5.）Clean test tube or Eppendorf tube

6.）Absorbent paper

7.）Automatic plate washer or8-tube pipettor

8.）ELISA data processing software. Recommend ELISACalc

9.）Beaker of 500ml and applicable measuring cylinder

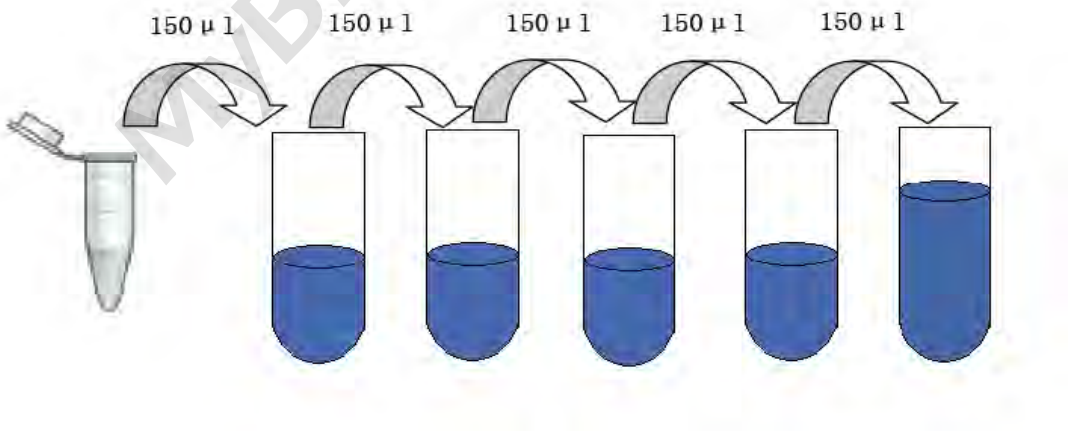
4. Preparation

a.Before using, put all the reagents in ambient temperature at least 30 minutes for stabilization.

b.It could be possible to add sample directly to this kit; if the concentration is too high, it could be diluted with proper proportion (recommend 2~5 times dilution); please multiply the result by the dilution times accordingly when calculation.

c.The cleaning solution is 25 times concentrated solution; please put the cleaning solution into 500ml or 1L beaker; and then metered to 500ml with double distilled water as operation solution.

d.Dilution of standard: take five clean Eppendorf tubes, add 150μl standard dilution into each tube, and marked respectively with 1200ng/L 600ng/L,300ng/L, 150ng/L, 75ng/L. Add 150μl standard stoste into the tube of 1200ng/L, take 150μl after blending and put into next tube until the last tube by that analogy. Zero well: add standard/ dilution of sample directly. (please refer to the figure below)



Stock2400ng/ml 1200ng/L 600ng/L 300ng/L 150ng/L 75ng/L

5. Treatment before sample

a.After the collection of sample, it shall extract as soon as possible

according to related documents, and then carry out the test as soon as possible. If the test cannot be carried out immediately, please reserve the sample with -20℃, and avoid thawing and refreezing regularly.

b.Serum: 10~20 minutes for natural coagulation in ambient temperature, and about 20 minutes for centrifugation (2000~3000 rpm). Collect serum carefully, and carry out centrifugation again if precipitation appears during reservation.

c.Blood plasma: choose EDTA or sodium citrate as anticoagulant according to requirements of sample; after blending with 10~20 minutes, centrifuge about 20 minutes (2000~3000 rpm). Collect supernatant carefully, and carry out centrifugation again if precipitation appears during reservation.

d.Urine: collect with aseptic tubes, centrifuge about 20 minutes (2000~3000 rpm). Collect supernatant carefully, and carry out centrifugation again if precipitation appears during reservation. The hydrothorax and cerebrospinal fluid shall refer to this.

e.Cell cultural supernatant: collect with aseptic tubes when detect its secretory. Centrifuge about 20 minutes (2000~3000 rpm). Collect supernatant carefully; when detect the compositions of cell, dilute cell suspension with PBS (PH7.2~7.4) until the concentration reaches around 1 million/ml. Destroy the cell to release its compositions by regularly thawing and refreezing. Centrifuge about 20 minutes (2000~3000 rpm).

Collect supernatant carefully, and carry out centrifugation again if precipitation appears during reservation.

f.Tissue: weight after cutting the sample. Add certain PBS with PH 7.4. And reserve it immediately with liquid nitrogen on cryopreservation. Keep 2~8℃ after melting the sample. And then add certain PBS (PH7.4), completely homogenate sample manually or with homogenizer. Centrifuge about 20 minutes (2000~3000 rpm). Collect supernatant carefully, keep one portion for inspection, and others for spare on cryopreservation.

6. Plate washing

a.Manually plate washing: wash away the remaining liquid in the enzyme-labeled well, and pat dry with absorbent paper; inject 300μl diluent washing solution into each well, throw away after 30s gently shaking, and then pat dry with absorbent paper. Repeat this process 4~5 times.

b.Automatic plate washing: automatic plate washer is quite convenient; and it should only be used in the test when you are quite familiar with its function and performance.

7. Detailed procedures

a.Stabilize the kit for half hour in ambient temperature before using.

b.Do not add sample into empty well, but chromogen solution A, B and stop solution for zero setting.

c.Standard well: add 50µl diluted standard into each well. Add 50µl standard/sample dilute into zero well, and then add 50µl enzyme-labeled reagent.

d.Sample well: add 50µl sample (recommend adding sample directly, and dilute 2~5 times with sample diluents if concentration is too high), then add 50µl enzyme-labeled reagent.

e.Shake gently after sealing with closure plate membrane, and then incubated 60 minutes at 37 ℃.

f.Dilute 25 times the 25×washing concentrate with distilled water for standby.

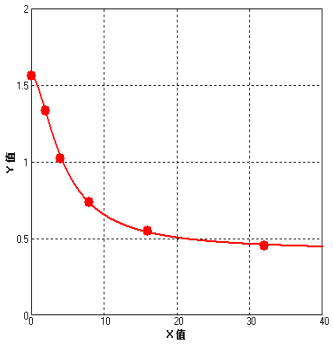
g.Washing: remove the membrane carefully, and drain the liquid, shake away the remaining water for drying. Add washing solution into each well, and discard it after stabilizing 30 seconds. Repeat the action 5 times, and then pat dry.

h.Chromogenic: add 50µl chromogenic solution A, then 50µl chromogenic solution B into each well. Gently shake to blending, incubate for 10 min at 37℃ away from light.

i.Stop: add 50µl stop solution into each well to stop the reaction(the blue changes into yellow immediately)

j.Final measurement: make zero setting by empty well, measure the optical density (OD) of each well under 450 nm wavelength which should be carried out within 10min after adding the stop solution.

k.Calculation: according to the concentration and OD values, calculate the standard curve linear regression equation with apply special calculation software; recommend to apply ELISAcalc for calculation, and to apply logistic curve for fitting model (four parameters). Please refer to the diagram below for standard curve:



8. Summary

Preparing of reagents, sample and standards

Add prepared sample and standards, enzyme-labeled reagent, reacting 60 minutes at 37 □

Plate washed five times, adding Chromogen solution A, B, reacting 10 minutes at 37 □

Add stop solution

Measure the OD value within 10min

Calculation

9. Performance and parameters

a.Tolerance within batch: CV＜10%

b.Tolerance between batches: CV＜12%

c.Sensitivity: 7.132ng/L

d.Preservation: 2-8℃

e..Specification: 96T/kit

f.Validity: 6 months (2-8℃)