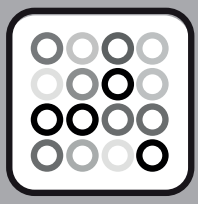
ELISA PathRF: Human TNF-α

3512-1HP-DP-1 | |

Datasheet & Protocol



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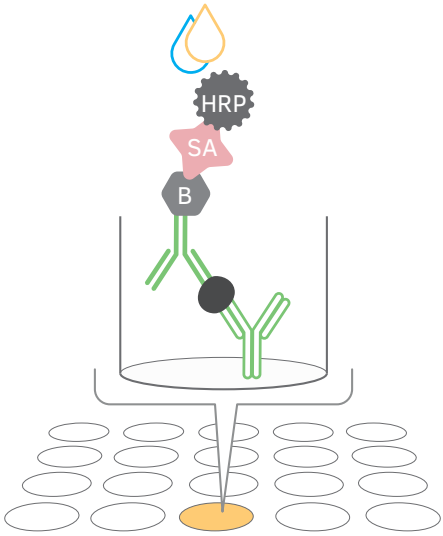
Introduction

ELISA PathRF for quantification of human TNF-α in plasma and serum samples containing rheumatoid factor (RF). For Research Use Only (RUO). Not for use in diagnostic procedures.

ELISA assay principle



ELISA PathRF kits are supplied with ELISA strip plates precoated with monoclonal antibody (mAb). Analyte in the sample is captured by the coated mAb and detected by the recombinant biotinylated detection mAb followed by Streptavidin-HRP (SA-HRP). Addition of TMB substrate will result in a colored substrate product. The reaction is stopped with sulfuric acid and the optical density can be quantified using an ELISA plate reader. The concentration of analyte is determined by comparison to a serial dilution of the ELISA standard analyzed in parallel.

Analysis of serum and plasma samples

ELISA PathRF kits are designed to eliminate potential interference from RF and heterophilic antibodies. The combination of a recombinant detection mAb and RF-block diluent prevents RF and heterophilic antibodies from cross-linking the assay antibodies, effectively reducing the risk of false-positive signal. RF and heterophilic antibodies can be found at high concentrations in serum and plasma from individuals with autoimmune diseases such as rheumatoid arthritis. The kit has been validated with RF-containing plasma.

Shipping and storage

The kit is shipped at ambient temperature. All reagents should be stored at 4-8 °C upon receipt, except the standard, which should be stored at -20 °C. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use. Do not combine components from different kit batches or components from other suppliers.

Contents

Component	
Pre-coated ELISA strip plate: Anti-TNF-α mAb MT25C5	1 x 96 wells
Recombinant human TNF-α ELISA standard	1 vial
Detection mAb MTR-9, biotin (0.5 mg/ml)	30 µl
Streptavidin-HRP	15 µl
Standard reconstitution buffer A5	1 ml
Wash buffer concentrate	120 ml
RF-block diluent	60 ml
Streptavidin-HRP diluent	15 ml
TMB substrate	15 ml
Stop solution	15 ml
Adhesive plate covers	3

To ensure total recovery of the stated quantity, bottles and vials have been overfilled.

Materials required but not supplied

- Microplate reader capable of reading at 450 nm
- ELISA plate washer; automated or manual (e.g., multipipette or squirt bottle)
- Precision pipettes, tips, and graduated cylinders
- Tubes for standard and sample dilutions
- Distilled or deionized water

Safety information

The Stop solution, 0.18 M H_2SO_4 (< 1%), is irritating to eyes and skin and should be handled with care. The standard should also be handled carefully as the effects of exposure are unknown. Buffers and reagents in solution contain the preservative Kathon CG (0.002%), a potential allergen that may cause sensitization through skin contact. Human and animal samples should be treated as potentially hazardous biologic material. All material should be disposed of in accordance with local regulations. For further information please consult the Safety Data Sheet on our website.

Preparation

- Allow the plates and assay reagents to reach room temperature before starting the assay (except for the TMB substrate which should preferably be used cold).
- Plan the plate layout to include a standard curve, samples, and an assay background control, all in duplicate. The volume per well should not exceed 100 µl. Include a plate blank (wells with only Substrate and Stop solution) to be used for subtraction before analysis.

Wash buffer

Add 50 ml Wash buffer concentrate to 950 ml distilled or deionized water (sufficient for all washing steps of 1 plate). If crystals have formed in the 20x concentrate, bring to room temperature and mix gently to dissolve.

Samples

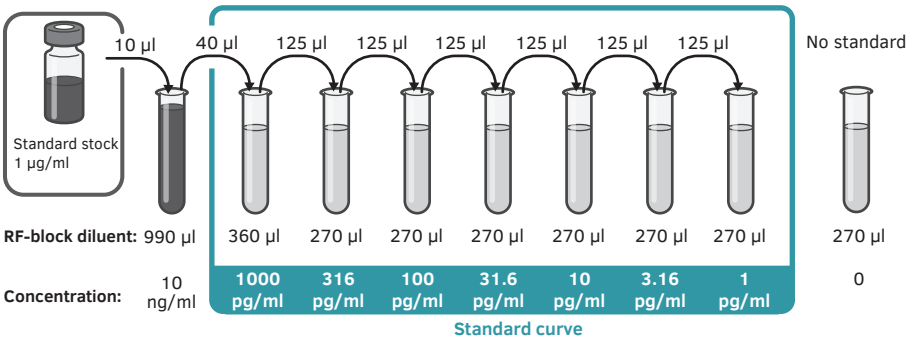
For accurate assay performance, all samples should be diluted at least 2-fold in RF-block diluent. Samples can be diluted in tubes or plates; diluent should be added before the samples and visible precipitates should be removed. The use of strongly hemolyzed and hyperlipemic samples may give inaccurate determination of the concentration. Samples containing high levels of analyte exceeding the standard range of the assay will require further dilution.

ELISA standard

Reconstitute the ELISA standard to a stock solution of 1 µg/ml by adding 1 ml Standard reconstitution buffer. Allow the standard to dissolve for 5 minutes and mix thoroughly. The standard should be kept in aliquots at -20 °C. Avoid repeated freeze-thaw cycles.

Preparation of standard curve

Dilute the standard stock solution to create a standard curve as shown. The indicated volumes are sufficient for duplicates. The last vial is used as an assay background control, i.e., the standard should be omitted. Prepare the standard curve within 30 minutes of use.



Detection antibody

Dilute the detection mAb in RF-block diluent to 1 µg/ml within 15 minutes of use. For each plate, add 24 µl detection mAb to 12 ml RF-block diluent.

Streptavidin-HRP

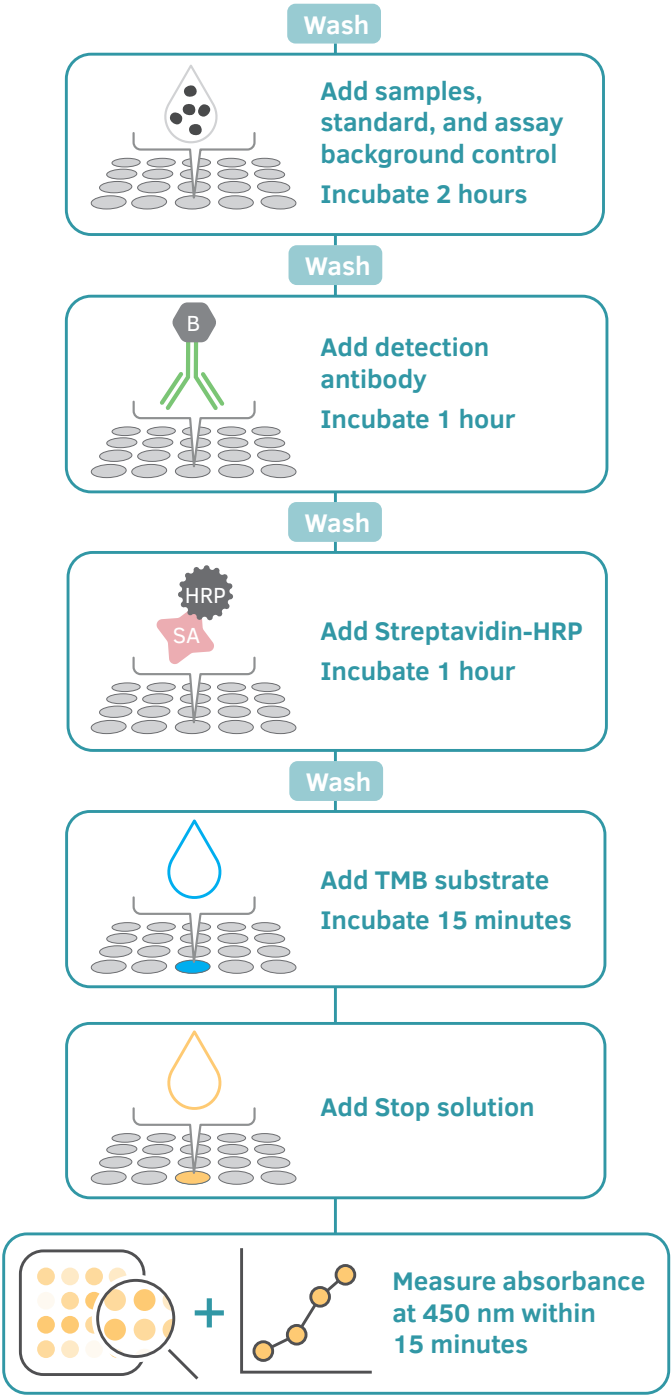
Dilute the Streptavidin-HRP 1:1000 in Streptavidin-HRP diluent within 15 minutes of use. For each plate, add 12 µl Streptavidin-HRP to 12 ml Streptavidin-HRP diluent.

Protocol

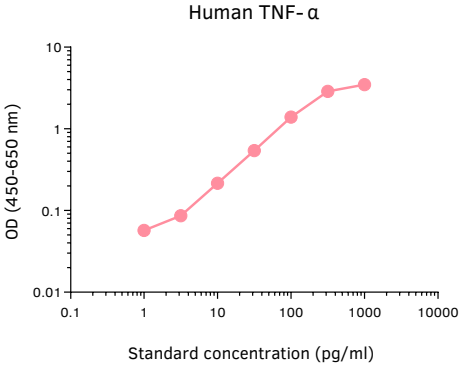
Prepare the reagents, standard curve, and samples as described in the Preparation section. Assemble the required number of strips in the plate frame and label the top of each strip. Store the remaining strips in the foil bag containing the desiccant at 4-8 °C.

1. Wash the plate 5 times with wash buffer, 300 µl per well. After the final wash, invert and tap the plate firmly against absorbent paper. Immediately proceed to the next step.
2. Add 100 µl per well of samples (diluted at least 2-fold), standard, and assay background control. Mix by tapping the plate. Cover the plate with an adhesive plate cover and incubate for 2 hours at room temperature.
3. Wash as in step 1.
4. Add 100 µl per well of detection mAb. Cover the plate and incubate for 1 hour at room temperature.
5. Wash as in step 1.
6. Add 100 µl per well of Streptavidin-HRP. Cover the plate and incubate for 1 hour at room temperature.
7. Wash as in step 1.
8. Add 100 µl of TMB substrate to each well. Incubate at room temperature, protected from direct light for 15 minutes.
9. Add 100 µl of Stop solution to each well to stop the color development.
10. Measure absorbance at 450 nm within 15 minutes. Preferably use a reader capable of subtracting a reference wavelength between 570 and 650 nm.

    We recommend the use of an ELISA software utilizing a 4- or 5-parameter curve fit. Subtract the mean absorbance value of the blank from the samples, standard and assay background control prior to creating the standard curve and analyzing the results.


Wash
Add samples, standard, and assay background control
Incubate 2 hours
Wash
B
Add detection antibody
Incubate 1 hour
Wash
HRP
SA
Add Streptavidin-HRP
Incubate 1 hour
Wash
Add TMB substrate
Incubate 15 minutes
Add Stop solution
Measure absorbance at 450 nm within 15 minutes

Performance



Representative standard curve

Standard range 3.16-1000 pg/ml

Sensitivity 2 pg/ml

The lowest concentration that can be detected, but not necessarily quantified with precision and accuracy. This was determined by adding 3 standard deviations to the mean OD of background wells.

Calibration

The standard of this ELISA has been calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. One ng equals 46 U of NIBSC standard 12/154. Please note that the calibration is batch specific.

Precision

	Intra-assay			Inter-assay		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/ml)	366.2	128.2	49.8	361.5	127.8	48.7
SD	25.5	10.0	3.1	9.1	4.2	3.5
CV (%)	7.0	7.8	6.3	2.5	3.3	7.2

Intra-assay and inter-assay precision were determined at 3 different concentrations of analyte (10 replicates per concentration in 3 assays).

Recovery

	Spike concentration (pg/ml)	Average recovery (%)
Plasma pool	350	85
	140	83
	56	87

A plasma pool from normal blood donors was spiked with standard (5 replicates per concentration in 3 assays). Average recovery is listed.

Linearity

A plasma pool from normal blood donors was spiked with a high concentration of standard and diluted (1:2 to 1:8) in RF-block diluent. Recovery ranged from 82 to 111%. RF positive control plasma (1900 IU/ml RF) was diluted (1:2 to 1:8) in RF-block diluent. The mid-curve recovery ranged from 95 to 114%.

Specificity

The kit is based on a matched pair of mAbs specific for native and recombinant human TNF-α.

MABTECH

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Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.

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