HUMAN TARC (CCL17) ELISA

Cat. No.: BA1032

For research use only!

Enzyme Immunoassay for the quantitative determination of TARC in human serum.
TARC (thymus and activation-regulated chemokine), also known as C-C motif chemokine ligand 17 (CCL17), is expressed in the thymus and is produced by dendritic cells, endothelial cells, keratinocytes, and fibroblasts[1]. TARC has affinity as a ligand for the C-C chemokine receptors CCR4 and CCR8 inducing Th2-dominant inflammatory reactions.

TARC has an important role in allergic diseases such as atopic dermatitis and bronchial asthma[2,3]. High serum concentrations of TARC are observed in patients with atopic dermatitis, and its concentration reflects the disease activity [4,5]. The concentration of TARC is also reported to be elevated in sputum of patients with bronchial asthma[6], and TARC may have potential as a therapeutic target. In clinical practice, TARC is useful also in selection of patients requiring new therapies of atopic dermatitis or asthma.[7]

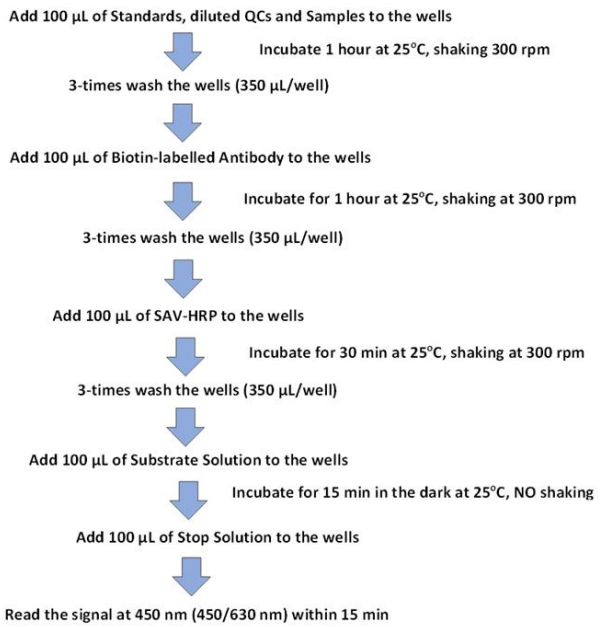
PRINCIPLE OF TARC ELISA

The microtiter plate is coated with the monoclonal antibody specifically binding TARC protein. The human serum is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well.

Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of TARC in the specimen. The concentration of TARC in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	*Qty.*
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard (lyophilized)	2 vials
Quality Control A (human serum, lyophilized)	2 vials
Quality Control B (human serum, lyophilized)	2 vials
Dilution Buffer	2x13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ± 2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ± 2°C.

Preparation of Standards

Reconstitute lyophilized human TARC Master Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human TARC in Master Standard is 2000 pg/mL.

Prepare set of Standard solution as follows:

Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 2 000 pg/mL (lyophilized)	See CofA	2 000 pg/mL
Std2	300 µL of Std1	300 µL	1 000 pg/mL
Std3	300 µL of Std2	300 µL	500 pg/mL
Std4	300 µL of Std3	450 µL	200 pg/mL
Std5	300 µL of Std4	300 µL	100 pg/mL
Std6	300 µL of Std5	300 µL	50 pg/mL
Std7	300 µL of Std5	450 µL	20 pg/mL
Blank	-	300 µL	0 pg/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls with 100 µL deionized/distilled water. Let the QCs rehydrate for 15 min and add 200 µL of Dilution Buffer.

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of **serum** is 1:3, i.e., for singlets 50 µL of sample + 100 µL of Dilution Buffer, for duplicates 100 µL of samples + 200 µL of Dilution Buffer, respectively.

Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for **30 min** at 25°C ±2°C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for **15 min**, at 25°C ±2°C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450 or 450/630 nm within 15 min.

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:3 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human TARC giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 4 pg/mL of sample.

2. Precision

Intra-assay

Sample	Mean (pg/mL)	SD	CV (%)
1	75	2.6	3.4
2	143	5.0	3.5

Inter-assay (Run – to – run)

Sample	Mean (pg/mL)	SD	CV (%)
1	81	4.7	5.8
2	249	15.8	6.3

3. Accuracy

Dilution linearity

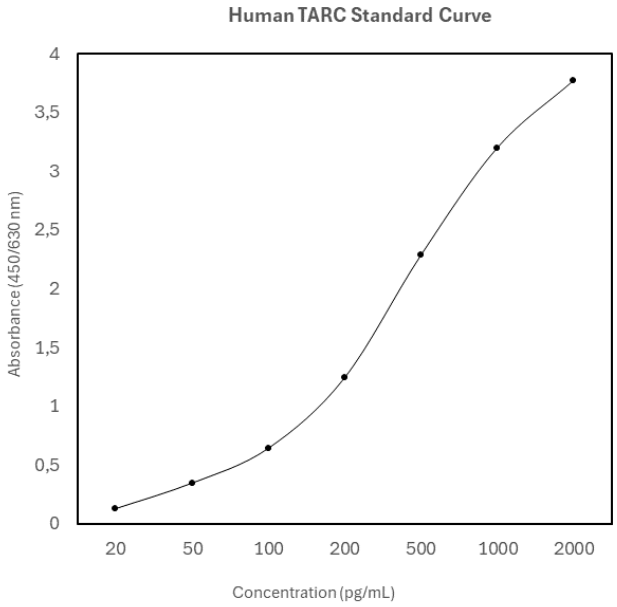
Sample	Dilution	Measured concentration (pg/mL)	Expected concentration (pg/mL)	Yield (%)
1	-	452	-	-
	2x	215	226	95
	4x	105	113	93
2	-	213	-	-
	2x	99	107	93
	4x	46	53	87

Spiking recovery

Sample	Spike (pg/mL)	Measured concentration (pg/mL)	Expected concentration (pg/mL)	Yield (%)
1	-	99	-	-
	500	509	599	85
	200	261	299	87
	100	182	199	91
2	-	34		
	500	441	534	83
	200	211	234	90
	100	115	134	86

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 40 unselected donors (26 men + 24 women) 21-65 years old were assayed with the Human TARC ELISA in our laboratory.

Age dependent distribution of TARC

Sex	Age (years)	n	TARC (pg/mL)				
			Mean	Median	SD	Min	Max
Men	21-65	26	136	118	94	25	286
Women	21-65	24	152	139	109	33	352

- **Reference range**

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological references ranges for TARC levels with the assay.

REFERENCES

1. Saeki, H. & Tamaki, K. Thymus and activation regulated chemokine (TARC)/CCL17 and skin diseases. J. dermatological Sci. 43, 75–84, (2006)
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3. Yamada A, Kiryu K, Takashino S, Yoshida M, Takeichi T, Kitamura O. Diagnostic value of serum thymus and activation-regulated chemokine (TARC) in fatal asthma. Forensic Sci Int. 365:112276 (2024)
4. Kakinuma, T. et al. Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity. J. allergy Clin. immunology 107, 535–541, (2001).
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7. Kataoka Y. Thymus and activation-regulated chemokine (CCL17) as a clinical biomarker in atopic dermatitis: significance and limitations in the new treatment era. Front Allergy 5:1473902 (2025)