ab325728 – Mouse TARC SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of TARC in mouse serum, plasma (EDTA), plasma (citrate), and cell culture supernatant.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab325728

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Limitations: All data, except typical standard curve, native serum and plasma, and sensitivity were collected using the colorimetric version of this kit (ab213474).

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Mouse TARC Capture Antibody 10X	600 µL	+4°C
Mouse TARC Detector Antibody 10X	600 µL	+4°C
Mouse TARC Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 4BR	6 mL	+4°C
Sample Diluent NBS	20 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
ChemiHRP Reagent A	3 mL	+4°C
ChemiHRP Reagent B	3 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

Sample Diluent NBS may contain precipitate, this is normal. If precipitate is not dissolved by gentle mixing, the precipitate may be dissolved by gentle warming and mixing at 37°C for 10 minutes. If precipitate remains, gently spin down and avoid visible precipitates when pipetting.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Sample Diluent 5BS (for plasma sample dilutions): Prepare Sample Diluent 5BS by diluting Sample Diluent NBS with Sample Diluent NS. To make 10 mL Sample Diluent 5BS combine 500 µL Sample Diluent NBS with 9.5 mL Sample Diluent NS. Mix thoroughly and gently.

Sample Diluent 50BS (for serum sample dilutions): Prepare Sample Diluent 50BS by diluting Sample Diluent NBS with Sample Diluent NS. To make 10 mL Sample Diluent 50BS combine 5 mL Sample Diluent NBS with 5 mL Sample Diluent NS. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BR. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BR. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

For serum and plasma samples, follow these instructions:

1. Reconstitute the **TARC** protein standard by adding the volume indicated on the protein vial label. For **serum samples measurements**, use Sample Diluent 50BS. For **plasma (citrate) and plasma (EDTA) samples measurements**, use Sample Diluent 5BS. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 2,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 80 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard 8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	320	80	2,000	1,600
2	Standard#1	75	150	1,600	533.3
3	Standard#2	75	150	533.3	177.8
4	Standard#3	75	150	177.8	59.3
5	Standard#4	75	150	59.3	19.8
6	Standard#5	75	150	19.8	6.6
7	Standard#6	75	150	6.6	2.2
8	Blank Control	75	150	0	0

For supernatant samples, follow these instructions:

1. Reconstitute the **TARC** protein standard by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes and mix thoroughly and gently. This is the 2,000 pg/mL **Stock Standard** Solution.
2. Label nine tubes, Standards 1– 9.
3. Add 80 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-9.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	320	80	2,000	1,600
2	Standard#1	75	150	1,600	533.3
3	Standard#2	75	150	533.3	177.8
4	Standard#3	75	150	177.8	59.3
5	Standard#4	75	150	59.3	19.8
6	Standard#5	75	150	19.8	6.6
7	Standard#6	75	150	6.6	2.2
8	Standard#7	75	150	2.2	0.7
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum*	≤ 25%
Plasma – Citrate*	≤ 50%
Plasma – EDTA*	≤ 50%
Lung Cell Culture Supernatant	3.13 – 50%

*Based on spiked sample.

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:4 into Sample Diluent 50BS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.
Plasma Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Dilute citrate samples at least 1:4 and EDTA samples at least 1:2 into Sample Diluent 5BS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma (heparin) samples.
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Assay, or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 30 minutes at room temperature on a plate shaker set to 750 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of mouse TARC.

The standard protein in this kit is full length mouse TARC.

Native signal was detected in serum, plasma (citrate), plasma (EDTA), and cell culture supernatant sample types.

Spiked protein experiments were used to validate serum, plasma (citrate), and plasma (EDTA) sample types.

Urine, saliva, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

This kit is incompatible with plasma (heparin) samples.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 25% serum samples of various species, interpolating the TARC protein concentrations from the mouse standard curve, and expressing the interpolated concentrations as a percentage of the TARC protein concentration in mouse serum assayed at the same dilution.

Reactivity was determined for the following species: Rat (64%)

Interpolated values were below the detectable range of the assay for the following species: Human

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	1,628	1,711	1,670
2.2	3,605	3,642	3,624
6.6	9,880	10,607	10,244
19.8	21,475	21,185	21,330
59.3	65,186	68,179	66,683
177.8	280,680	267,950	274,315
533.3	1,035,700	1,058,300	1,047,000
1,600	3,604,500	3,674,200	3,639,350

Table 1. Example of mouse TARC standard curve in Sample Diluent 50BS. The TARC standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	2,765	2,681	2,723
2.2	4,334	4,306	4,320
6.6	11,058	11,134	11,096
19.8	22,359	26,795	24,577
59.3	88,592	97,546	93,069
177.8	339,180	353,130	346,155
533.3	1,357,100	1,342,600	1,349,850
1,600	4,516,700	4,512,800	4,514,750

Table 2. Example of mouse TARC standard curve in Sample Diluent 5BS. The TARC standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	838	869	854
0.7	1,413	1,395	1,404
2.2	2,830	2,928	2,879
6.6	7,877	7,833	7,855
19.8	20,168	20,010	20,089
59.3	86,873	82,281	84,577
177.8	330,170	311,710	320,940
533.3	1,393,100	1,348,100	1,370,600
1,600	5,058,900	5,014,300	5,036,600

Table 3. Example of mouse TARC standard curve in Sample Diluent NS. The TARC standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent 50BS	16	1.01 pg/mL
Sample Diluent 5BS	16	1.12 pg/mL
Sample Diluent NS	16	0.50 pg/mL

Recovery

Three concentrations of TARC were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
25% Mouse Serum	86	81 – 92
50% Mouse Plasma – Citrate	101	95 – 107
50% Mouse Plasma – EDTA	107	103 – 112
50% Mouse Lung Supernatant	98	93 – 101

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Recombinant TARC was spiked into the following biological samples and then diluted in a 2-fold dilution series. Serum sample dilutions are made in Sample Diluent 50BS. Plasma sample dilutions are made in Sample Diluent 5BS.

Dilution Factor	Interpolated value	25% Mouse Serum	50% Mouse Plasma (Citrate)	50% Mouse Plasma (EDTA)
Undiluted	pg/mL	328	744.4	824.7
	% Expected value	100	100	100
2	pg/mL	170	368.3	414.7
	% Expected value	104	99	101
4	pg/mL	77	168.4	183.2
	% Expected value	94	90	89
8	pg/mL	38	81.8	88.5
	% Expected value	94	88	86
16	pg/mL	17	38.8	40.5
	% Expected value	82	83	79

Native TARC was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent 50BS.

Dilution Factor	Interpolated value	50% Mouse Lung Supernatant
Undiluted	pg/mL	424.0
	% Expected value	100
2	pg/mL	199.3
	% Expected value	94
4	pg/mL	95.7
	% Expected value	90
8	pg/mL	43.9
	% Expected value	83
16	pg/mL	20.9
	% Expected value	79

Precision

Mean coefficient of variations of interpolated values of TARC from three concentrations of mouse lung supernatant within the working range of the assay.

	Intra-assay	Inter-assay
N=	5	3
CV (%)	9.6	6.8

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
https://www.abcam.com/en-us/technical-resources/guides/elisa-guide

Technical Support

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