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BIOSCIENCES

# **Tyrosine Phosphatase-related Islet Antigen 2 AutoAb ( IA2A ) ELISA Assay Kit**

Catalog Number: TPI31-K01 ( 1x 96 Wells )

For Research Use Only

*v. 1.0 ( effective 06 SEP 2023 )*

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## **INTENDED USE**

The Eagle Biosciences Tyrosine Phosphatase-related Islet Antigen 2 AutoAb (IA2A) ELISA Assay Kit is a highly sensitive and specific ELISA kit for precision detection and quantitative measurement of IA-2 autoantibody (IA2A) titres in human serum (plasma samples are not recommended). This kit is for research use only and not for use in diagnostic procedures.

*For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at [www.EagleBio.com](http://www.EagleBio.com) or at 866-411-8023.*

## **ASSAY BACKGROUND**

The prevalence of autoantibodies against protein tyrosine phosphatase 2 (IA-2/ICA512) is 55-75% in newly onset Type I diabetes (T1D) patients. T1D patients who possess autoantibodies against IA-2 have a higher risk of faster T1D development. IA-2 autoantibodies detection is able to increase the detection rate of T1D to 94%[1]. Besides T1D, IA-2 autoantibodies also exist in autoimmune thyroid diseases (AITD) [2].

## **ASSAY PRINCIPLES**

In the IA2A ELISA, recombinant IA-2 protein coated onto plate wells can specifically recognize the IA-2 autoantibodies in human sera and calibrators. After a 16–20-hour incubation at 4°C, IA-2 autoantibodies are captured by immobilized IA-2 protein while the excess or unbound components were discarded and washed away. Afterwards, biotinylated IA-2 protein (IA2-Biotin) is added for another round of incubation for 1 hour at 4°C, wherein the IA2-Biotin detects IA-2 autoantibodies previously bound to IA-2 protein on the plate. After removal of nonspecific bindings, bound IA2-Biotin is revealed by addition of streptavidin horseradish peroxidase (STV-HRP), which specifically binds with biotin, followed by the substrate 3,3',5,5'-Tetramethylbenzidine (TMB), which results in formation of a blue color. Color reaction will be further stopped by 2M H<sub>2</sub>SO<sub>4</sub>, transforming the blue color to yellow signals. The absorbance of yellow reaction mixture is measured by plate reader at 450nm and 405nm. The higher the reading is, the higher concentration of IA-2 autoantibodies. Low concentration of IA-2 autoantibodies (<15 u/ml) is recommended to be read off the 450nm calibration curve, while high value of IA-2 autoantibodies to be read off 405nm standard curve. The measuring interval is 5-2000U/ml (units are NIBSC 97/550)

## **STORAGE AND PREPARATION OF TEST SERUM SAMPLES**



Test samples are suggested to be assayed immediately after separation of serum, or preferably stored at -200C or below in aliquots. Duplicate test is recommended therefore 50µl is sufficient for each aliquot (25µl per test). Lipemic or hemolyzed sera, as well as plasma samples are not recommended. When required, vortex test serum samples at room temperature to ensure homogeneity. Then centrifuge samples at 10-15,000 rpm for 5 minutes prior to assay to remove particulates. Please do not omit this centrifugation step if samples are cloudy and containing particles.

**MATERIALS NEEDED BUT NOT SUPPLIED**

1. Pipettes capable to dispense 1000 µl, 100 µl, and 25 µl
2. Multi-channel pipettes
3. Plate shake capable of shaking at 500 rpm.
4. 96-well plate reader capable of absorbance measurement at 450 nm and 405 nm
5. Distilled water

**REAGENTS PROVIDED**

A	IA-2 protein coated ELISA plate	12 strips of 8 wells (96 wells in total) in a frame and sealed in a foil bag. Make sure test strips are firmly fitted into the provided frame before use. Equilibrate test strips to room temperature before use. After opening, seal unused strips in the original self-seal foil bag with desiccant. Store the re-sealed foil bag at 2-8C for up to 16 weeks.
B 1-7	Calibrators	1ml x 6 - 7.5, 15, 35, 120, 350, 4000 ( U/ml) ( Units are WHO standard NIBSC 97/550) Ready to use
C	Positive control	1 ml x 1 - Ready to use
D	Negative control	1 ml x 1 - Ready to use
E	IA2-Biotin	3 vials Lyophilized
F	Reconstitution buffer	15ml x 1 - Pale yellow



		Ready-to-use for reconstitution of IA2-Biotin.
G	20 x Streptavidin horseradish peroxidase (STV-HRP)	1ml x 1 - Dilute 1 in 20 with dilution buffer (H). For example, 0.5 mL (G) + 9.5 mL (H).
H	Dilution buffer (for STV-HRP)	15ml x 1 - Ready to use
I	Substrate solution (3,3',5,5'-Tetramethylbenzidine, TMB)	Ready to use - Equilibrate to room temperature for 15 minutes before use
J	Stop solution	15ml x 1 2M H <sub>2</sub> SO <sub>4</sub> - Ready to use
K	10X Wash buffer	50ml x 1 - Dilute with distilled water (1:10)

## ASSAY PROCEDURE

### PREPARATION OF REAGENTS

1. 1X wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 ml) with 450 ml of distilled water or deionized water. If precipitates are observed in the 10× Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

2. 1x detection IA2-Biotin solution

Reconstitute each vial of lyophilized IA2-Biotin with 4.5ml cold IMD reconstitution buffer (F). Return the reconstituted detection solution to 2-8°C immediately. Please freshly prepare the reconstituted IA2-Biotin solution prior adding to the assay.

3. 1x STV-HRP solution

Dilute 1 in 20 with dilution buffer (H). For example, 0.5 mL (G) + 9.5 mL (H). The 1×STV HRP buffer may be stored at 2-8°C for up to one month.

**Please pre-balance all the reagents to room temperature (20-25°) except IA2-Biotin (E) and reconstitution buffer (F) for at least 30 minutes before use.**



<b>Step 1</b>	Pipette 25µl of test serum samples, calibrators ( B1-7 ) and controls ( C and D ) into respective plate wells in duplicate. Leave at least one well for blank.
<b>Step 2</b>	Cover the frame and shake the wells at room temperature at 500 rpm for 30s on a plate shaker. Then place the plate at 4°C, without shaking, for 16-20 hours.
<b>Step 3</b>	Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
<b>Step 4</b>	Add 100 µl of reconstituted GAD-Biotin to each well.
<b>Step 5</b>	Cover the frame, shake the wells at 500 rpm for 5s on a plate shaker and place the wells at 4°C for 1 hour without shaking.
<b>Step 6</b>	Wash each well 3 times as described in step 3.
<b>Step 7</b>	Pipette 100 µl of 1x STV-HRP solution to each well
<b>Step 8</b>	Cover the frame and shake the wells at room temperature at 500 rpm for 20 minutes on a plate shaker.
<b>Step 9</b>	Wash each well 4 times as described in step 3.
<b>Step 10</b>	Add 100 µl of Substrate solution ( I ) to each well, incubate at room temperature for 15 minutes. <b>Protect from light.</b>
<b>Step 11</b>	Add 100 µl of Stop solution ( J ) to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
<b>Step 12</b>	Measure absorbance of each well at 450 nm and/or 405 nm immediately.

## DATA ANALYSIS

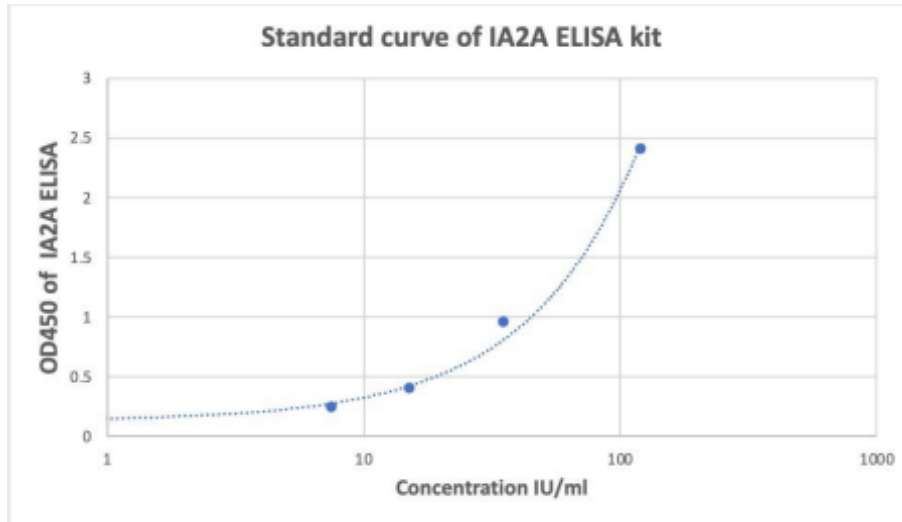


1. Subtract absorbance of blank from that of standards and samples.
2. A calibration curve can be established by plotting calibrator concentration on x-axis (log-scale) against the absorbance of the calibrators on the y-axis (linear scale). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-lin curve fitting can be used for calculation.
3. The IA-2 autoantibody concentrations in sera can be read off the calibration curve. Negative control can be assigned a value of 0.5U/ml to assist the statistical software to process the data analysis. Samples with high IA-2 autoantibody concentrations can be diluted with IA-2 autoantibody negative serum or the kit negative control. Some sera will not dilute in a linear way according to the kit calibrators (standardized against NIBSC 97/550). Most test sera will have values below 350 u/mL and the 2000 u/mL calibrator need not always be included.

## TYPICAL RESULTS

*Example only, not for calculation of actual results.*

Calibrator *	Concentration (IU/ml)	OD450	OD405
1	4000	8.804	2.574
2	350	5.9626	1.743
3	120	2.107	0.619
4	35	0.771	0.226
5	15	0.371	0.109
6	7.5	0.231	0.067
Negative Control	0	0.079	0.023
Positive Control	40	1.033	0.328



**ASSAY CUT-OFF VALUE**

< 7.5 IU/ml	Negative
≥ 7.5IU/ml	Positive

This cut-off value has been validated. However, it is recommended that each laboratory should establish its own normal and pathological reference range for IA2 autoantibody level. Furthermore, it is also recommended that each laboratory should include its own panel of control samples in the assay.

**CLINICAL EVALUATION**

<b>Clinical Evaluation</b>		
Sensitivity	60% (n=40)	
Specificity	96.1% (n=77)	
<b>Inter Assay Precision</b>		
Sample	IU/ml (n=6)	CV
1	360	2.42%
2	40	5.1%



3	7.5	6.22%
<b>Intra Assay Precision</b>		
Sample	IU/ml ( n=20 )	CV
1	360	2.10%
2	40	5.91%
3	7.5	6.68%

## REFERENCE

1. Leslie, R.D., M.A. Atkinson, and A.L. Notkins, Autoantigens IA-2 and GAD in Type I (insulin-dependent) diabetes. *Diabetologia*, 1999. 42( 1 ): p. 3-14.
2. Nakamura, H., et al., Prevalence of interrelated autoantibodies in thyroid diseases and autoimmune disorders. *Journal of endocrinological investigation*, 2008. 31(10): p. 861-865.

## PRECAUTIONS

- This kit is for research use only
- Compare contents and packing list, if there is breakage or shortage, notify Eagle Biosciences immediately
- Do not pipette reagents by mouth
- Do not smoke, eat or drink while performing assay
- Wear disposable gloves and proper lab protection and attire
- Treat all samples as potentially infectious
- Do not mix reagents from other lots
- Avoid contact with TMB and Stop solutions. If contact occurs, rinse thoroughly with water
- Eagle Biosciences is not responsible for outcomes as results of tampering with the reagents or using them unconventionally

## WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This





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For further information about this kit, its application, or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.