Deoxyribonuclease B Antibody (Human) ELISA Kit (OKCA00412)

Instructions for use

For the quantitative measurement of Deoxyribonuclease B Antibody in serum.

This product is intended for research use only.

Lot to lot kit variations can occur. Use the kit manual which has been provided along with the kit packaging.

Table of Contents

1. Background

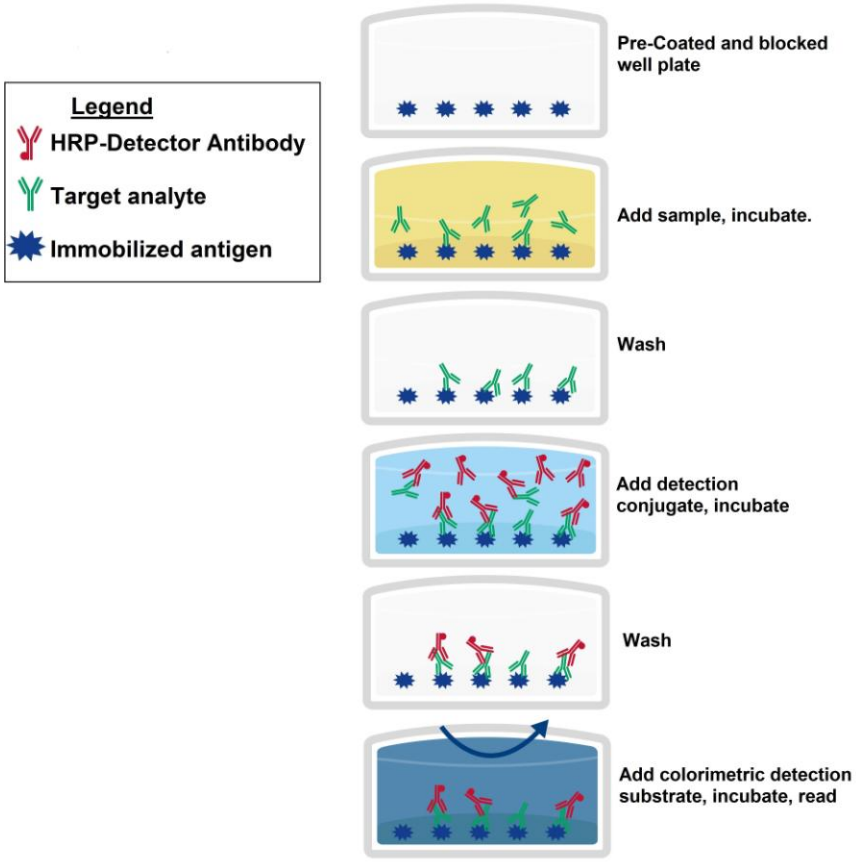
Principle

Aviva Systems Biology Deoxyribonuclease B Antibody (Human) ELISA Kit (OKCA00412) is based on standard reverse capture sandwich enzyme-linked immuno-sorbent assay technology. Deoxyribonuclease B antigen has been pre-coated and blocked in a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and washed. An HRP conjugated detector antibody specific for Human Antibody is added, incubated and followed by washing. An enzymatic reaction is produced through the addition of substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration is read by absorbance at 450 nm and is quantitatively proportional to the amount of sample Deoxyribonuclease B Antibody captured in well.

General Specifications

General Specifications	
Specificity	Human Deoxyribonuclease B Antibody
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Upon receipt store kit at 4°C for 6 months.

4. Kit Components

•The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Deoxyribonuclease B Antibody Microplate	96 Wells (12 x 8 Well strips)	4°C for 6 Months
Negative Control	1 x 300 µL	
Positive Control	1 x 300 µL	
Anti-Human Antibody HRP-Conjugate	1 x 12 mL	
Sample Diluent	1 x 12 mL	
30X Wash Buffer	2 x 15 mL	
Substrate A	1 x 6 mL	
Substrate B	1 x 6 mL	
Stop Solution	1 x 6 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.

• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.

• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.

• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.

• Automated plate washer (optional).

• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.

• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.

• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.

• Absorbent paper or paper toweling.

• Distilled or deionized ultrapure water.

• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.

• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.

• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.

• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.

• Replicate wells are recommended for standards and samples.

• Cover microplate while incubating to prevent evaporation.

• Do not allow the microplate wells dry at any point during the assay procedure.

• Do not reuse tips or tube to prevent cross contamination.

• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.

• Completely remove of all liquids when washing to prevent cross contamination.

• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.

• Equilibrate all materials to ambient room temperature prior to use (standards exception).

• For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.

• Pipetting less than 1 µL is not recommended for optimal assay accuracy.

• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.

• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

• Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.

• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use prepare immediately prior to use.

• The following reagents are provided at ready to use concentrations and require no preparation:

- Negative Control
- Positive Control
- Anti-Human Antibody HRP-Conjugate
- HRP-Conjugate diluent
- Sample Diluent
- Substrate A
- Substrate B
- Stop Solution

8.1 1X Wash Buffer

8.1.1 If crystals have formed in the 30X **Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

8.1.2 Add the entire 15 mL contents of the 30X **Wash Buffer** bottle to 435 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

8.1.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.1.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.2 Microplate Preparation

• Micro-plates are provided ready to use and do not require rinsing or blocking.

• Unused well strips should be returned to the original packaging, sealed and stored at 4°C.

• Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

• Store samples to be assayed at 2-8°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C.Avoid repeated freeze-thaw cycles.

• Samples not indicated in the manual must be tested to determine if the kit is valid.

• Prepare samples as follows:

• Serum - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

9.2 Sample Dilution (1:10)

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

• Dilute samples using **Sample Diluent**.

• Mix diluted samples gently and thoroughly.

• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

Serum samples require a 10-fold dilution into normal saline before test. The suggested 10-fold dilution can be achieved by adding 10µl sample to 90µl of normal saline.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for inter- and intra-assay reproducibility will be observed when incubation at 37°C as indicated in the procedure below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Assign a well for an absolute blank.

10.3 Add 100 µL of **Sample Diluent** into wells of the **Deoxyribonuclease B Antibody Microplate**.

10.4 Immediately add 10 µL of the **1:10 diluted samples**, **Positive Control** or **Negative Control** to test wells. At least two replicates of each control, sample or blank is recommended.

10.5 Mix gently and thoroughly by tapping the plate.

10.6 Cover the plate with the well plate lid and incubate at 37°C for 45 minutes.

10.7 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.8 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.9 Wash plate 5 times with **1X Wash Buffer** as follows:

10.9.1 Add 300 µL of **1X Wash Buffer** to each assay well.

10.9.2 Incubate for 30 seconds.

10.9.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.9.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.9.5 Repeat steps 10.7.1 through 10.7.4 **four** more times.

10.10 Add 100 µL of the **Anti-Human Antibody HRP-Conjugate** to all the wells.

10.11 Cover with the well-plate lid and incubate at 37°C for 30 minutes.

10.12 Remove the plate lid and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.13 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.14 Repeat the wash in step 10.9 with **five** washes.

10.15 Add 50 µL of **Substrate A** and 50 µL of **Substrate B** well and incubate at 37°C **in the dark** for 10 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.16 Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done in step 10.15. Gently tap the plate to mix.

10.17 Read the O.D. absorbance at 450 nm with a standard microplate reader within 10 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 540 nm or 570 nm.

11 Calculation of Results

A positive or negative Deoxyribonuclease B Antibody determination is derived by comparing the test samples to the **Positive** and **Negative Controls**.

(1) Negative Control OD values must nomore than 0.1.

If one of the Negative Control OD values high than 0.1, discard it.

If two or more than two Negative Control OD values high than 0.1, repeat the test.

If the average value of ODnegative＜0.05, calculate it as 0.05.

(2) Positive Control OD Values must no less than 0.6.

If one of the Positive Control OD values less than 0.6, discard it.

If two Positive ControlOD valuesless than 0.6, repeat the test.

(3) A cutoff value was defined as the average Negative Control value plus 0.2.

While ODsample＜Cutoff Value: Negative

While ODsample≥Cutoff Value: Positive

12 Typical Expected Data

12.15 Reproducibility

Intra-assay Precision: 3 samples with known low, middle and high levels Deoxyribonuclease B Antibody were tested with 20 replicates on one plate, respectively.

Inter-assay Precision: 3 samples with known low, middle and high level Deoxyribonuclease B Antibody were tested on 3 different plates, 8 replicates in each plate.

Mean Intra-Assay: CV ≤15%

Mean Inter-Assay: CV ≤15%

13 Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

Aviva Systems Biology, Corp.
5754 Pacific Center Blvd, Suite 201
San Diego, CA 92121

Phone: 858-552-6979
Toll Free: 888-880-0001
Fax: 858-552-6975

Technical support: techsupport@avivasysbio.com

China

Beijing AVIVA Systems Biology
6th Floor, B Building, Kaichi Tower
#A-2 Jinfu Road.
Daxing Industrial Development Zone
Beijing, 102600, CHINA

Phone: (86)10-60214720
Fax: (86)10-60214722
E-mail: support@avivasysbio.com.cn

中国地址：北京大兴工业开发区金辅路甲 2 号凯驰大厦 B 座 6 层 (102600)
电话: 010-60214720/21
传真: 010-60214722

产品售前咨询及销售: sales@avivasysbio.com.cn
售后及技术支持: support@avivasysbio.com.cn