RayBio® Custom Indirect Human IgG ELISA Kit

Catalog #: IE-Custom-IgG

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Caution:
Extraordinarily useful information enclosed

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RayBiotech, Inc.

RayBio® Custom Indirect Human IgG ELISA Kit

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Please read the entire manual carefully before starting your experiment

I. INTRODUCTION

The Custom Indirect Human IgG ELISA Kit is an in vitro indirect ELISA for the measurement of human IgG antibody against custom antigen(s) in human serum or plasma. This ELISA kit is for research use only, not for therapeutic or diagnostic applications.

PRINCIPLE OF THE ASSAY

This custom human antibody IgG ELISA kit employs an indirect ELISA method, seen in Figure 1. In this kit, standard 96-well plates (12 strips with 8 wells/strip) are coated with a custom protein, which combines with the corresponding antibody present in a sample. When a biotinylated secondary anti-human IgG antibody is added, a complex forms between the biotinylated secondary antibody and human antibody bound to the protein coated plate. An HRP-S solution is added, which complexes with the biotinylated secondary antibody. A TMB substrate is then added, and a blue color is generated. The depth of color is relative to the amount of the human anti-custom antigen antibody present. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

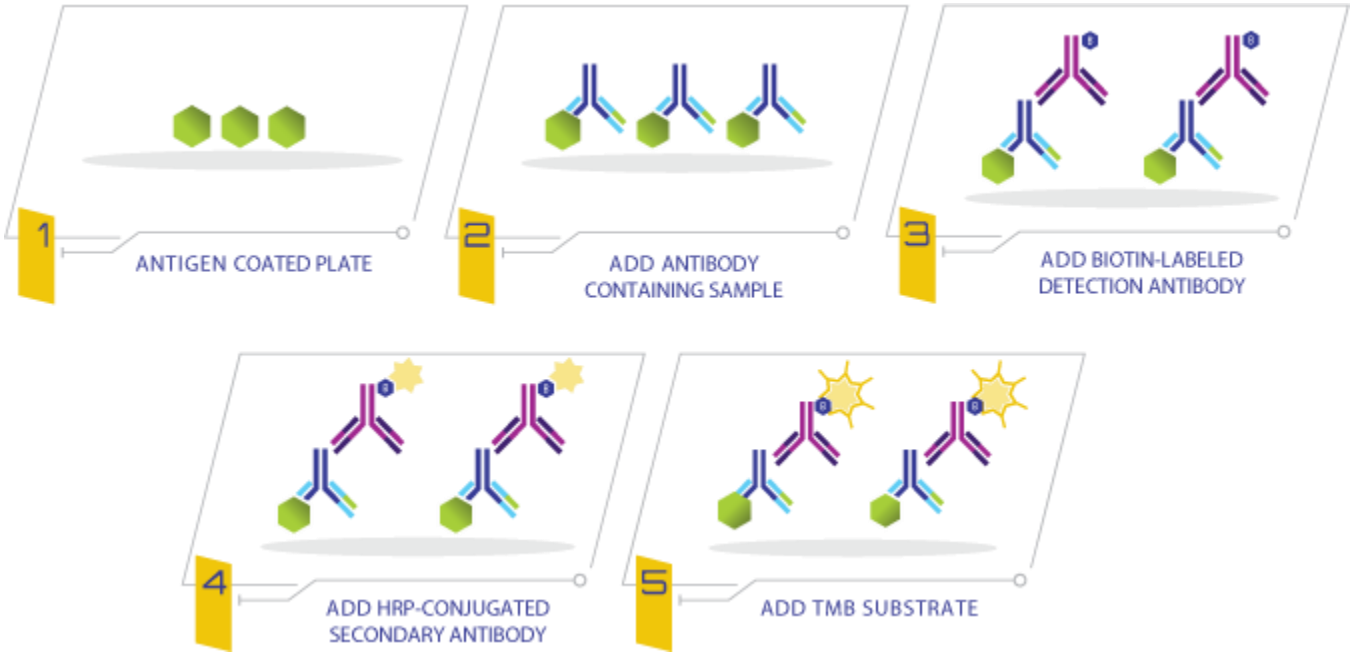

Figure 1. Indirect ELISA method

II. REAGENTS AND STORAGE

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

Item	Component	SIZE	STORAGE AFTER PREPARATION*
A	Uncoated 96-well Microplate**	1 plate	Room Temperature
B	20x Wash Buffer Concentrate	1 vial (25 mL)	4ºC
C	1x Coating Buffer	1 vial (25 mL)	
D	Blocking Buffer	1 vial (25 mL)	
E	5x Assay Diluent Concentrate	1 vial (15 mL)	
F	Biotinylated Anti-Human IgG	1 vial (15 µl)	4ºC (5 days)
G	HRP-Streptavidin concentrate	1 vial (70 µl)	Do not store and reuse.
H	TMB One-Step Substrate Reagent	1 vial (12 mL)	4ºC
I	Stop Solution***	1 vial (8 mL)	
J	5X Sample Diluent	1 vial (25 mL)	

*For up to 1 month (unless otherwise stated) or until expiration date.
**Store at 4ºC after coating (see section V).
***Contains 0.2 M Sulfuric Acid.

III. REAGENT PREPARATION

NOTE: Thaw all reagents to room temperature immediately before use. If wash buffers contain visible crystals, warm to room temperature, and mix gently until dissolved.

NOTE: Briefly centrifuge ITEMS F and G before opening to ensure maximum recovery.

Item	Component	Preparation	Example
A	Uncoated 96-well Microplate	See section V.	
B	20x Wash Buffer Concentrate	Dilute 20-fold with deionized water	25 mL concentrate with 475 mL water
C	1x Coating Buffer	No preparation	N/A
D	Blocking Buffer	No preparation	N/A
E	5x Assay Diluent Concentrate	Dilute 5-fold with deionized water	20 mL concentrate with 80 mL water
F	Biotinylated Anti-Human IgG*	Add 200 µl 1X Assay Diluent B to prepare 100X antibody concentrate Dilute concentrate 100-fold with 1x Sample Diluent*	100 µl of antibody concentrate and 9900 µl 1x Assay Reagent
G	HRP-Streptavidin concentrate*	Dilute 800-fold with 1x Assay Diluent*	10 µl of antibody and 7990 µl of 1x Assay Diluent
H	TMB One-Step Substrate Reagent (Item H)	No preparation	N/A
I	Stop Solution (Item I)	No preparation	N/A
J	5X Sample Diluent (Item J)	Dilute 5-fold with deionized water	20 mL concentrate with 80 mL water

*Dilutions for ITEMS F and G are given as recommended values but will need to be determined by the researcher for each assay.

IV. ADDITIONAL MATERIALS REQUIRED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.

V. MICROPLATE COATING PROCEDURE

1. Dilute custom coating protein to a concentration of 0.5 µg/mL in Coating Buffer (Item C). Mix thoroughly and add 100 µl to each well of the uncoated 96-well plate. Gently rock the plate for 15 minutes at room temperature, transfer to 4°C, and leave the plate to gently rock overnight.

   Note: Protein concentration provided here is only a recommended value. The optimal concentration will need to be empirically determined by the researcher for each protein.

2. Discard the solution and wash 4 times with 1X Wash Buffer (Item B). Wash by filling each well with 300 µl of 1X Wash Buffer using a multi-channel Pipette or autowasher. Complete removal of all liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

3. Apply 120 µl blocking buffer (Item D) to each coated well, and gently rock at room temperature for 90 minutes.

4. Discard blocking solution over sink and blot against clean paper towels to remove excess blocking buffer. Let the plate airdry at room temperature for 1 hour before use.

   Note: Ensure plate is protected from dust during this step. Covering the plate lightly with a single kimwipe layer provides dust protection while allowing for adequate air flow.

VI. ASSAY PROCEDURE

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended that the positive control, and all samples be run at least in duplicate.

2. Label removable 8-well strips as appropriate for your experiment.

3. Prepare sample by dilution as required in 1X Sample Diluent (Item E). Add 100 µl of each prepared sample into appropriate wells. Cover wells and incubate for 1 hour at room temperature with gentle shaking.

   Note: The required sample dilution will vary. The optimum dilution will need to be empirically determined by the researcher for each protein and sample type.

4. Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with 300 µl of 1X Wash Buffer using a multi-channel Pipette or autowasher. Complete removal of all liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µl of prepared biotinylated anti-human IgG antibody (Item F, Section III) to each of the sample wells. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µl of prepared HRP-Streptavidin solution (Item G, Section III) to each well. Incubate for 30 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 15-30 minutes at room temperature in the dark with gentle shaking.

   Note: Incubation time may vary depending on protein and sample type, see VIII Trouble Shooting Guide for more information.

10. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.

2. Add 100 µl sample to each well. Incubate 1 hour at room temperature.

3. Add 100 µl prepared Biotinylated Anti-Human IgG Antibody into respective wells. Incubate 1 hour at room temperature.

4. Add 100 µl prepared HRP-Streptavidin solution to each well. Incubate 30 minutes at room temperature.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 15-30 minutes at room temperature.

6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

VIII. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Low signal	1. Improper preparation of sample, antibody, or HRPS	Briefly spin down vials before opening.
	2. Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation.
	3. Insufficient incubation times	Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background).
Large CV	1. Inaccurate pipetting	Check pipettes.
	2. Air bubbles in wells	Remove bubbles in wells.
High background	1. Plate is insufficiently washed	Review the manual for proper wash procedure. If using a plate washer, ensure that all ports are unobstructed.
	2. Contaminated wash buffer	Make fresh wash buffer.
Signal Saturation	1. Incubation times are too long	Reducing the incubation time of TMB step most often resolves this issue. Other incubation times can also be adjusted.
	2. Reagents too concentrated	Sequentially reduce concentration of sample, antibody, or HRPS

This product is for research use only.