DRG® 90 IgG Foods ELISA (EIA-1745)

Revised 01 Nov. 2011 rm (Vers. 3.1) **USA: RUO**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

INTENDED USE

The 90 Food IgGElisa Test is for measuring the relative amount of food-specific IgG antibody in human serum.

PRINCIPLE OF THE TEST

Specific allergens are immobilized separately onto microtitre wells. The allergens are allowed to react with specific antibodies present in the donor's serum. Excess serum proteins are removed by the wash step. Enzyme labeled antibody conjugate is allowed to react with allergen-antibody complex. A color is developed by the addition of a substrate that reacts with the coupled enzyme. The color intensity is measured and is directly proportional to the concentration of IgG antibody specific to a particular allergen.

REAGENTS AND MATERIALS

This test kit contains sufficient wells and reagents to assay 3 donor sera for antibodies to 90 different foods.

PLA FOOD	= Food Extract Coated Microwell Plates.	3 plates
DIL SPE 1X	= Sample Diluent (Green)	1 x 56 mL
BUF WASH 66.67X	= Wash Buffer (concentrate)	1 x 30 mL
CAL FOOD IgG	= Food IgG Calibrator	1.0 mL
CTRL + IgG	= Food IgG Positive Control	1.0 mL
CONJ ENZ IgG-HRP	= Food IgG-HRP Conjugate	1 x 40 mL
SUBS A TMB	= Substrate Solution A (TMB)	2 x 12 mL
SUBS B H2O2	= Substrate Solution B (hydrogen peroxide)	2 x 12 mL
SOLN STOPPING	= Stopping Solution (1N H_2SO_4)	1 x 20 mL

WARNINGS AND PRECAUTIONS

1. Potential Biohazardous Material

The source of the Calibrators and Controls is human serum. The human serum used has been found non-reactive to HbsAg, anti-HIV 1/2 and anti-HCV when tested with FDA licensed reagents. Because there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled as if potentially infectious

2. Sodium Azide

Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper or brass to form explosive metal azides. When disposing of these materials, always flush with large volumes of water to prevent azide buildup.

3. Stopping Solution

Stopping Solution consists of 1N H2SO4. This is a strong acid and should be handled with caution. It can cause burns and should be handled with gloves. Wear eye protection and appropriate protective clothing. Avoid inhalation. Dilute a spill with water before absorbing the spill with paper towels.

PREPARATION OF DONOR SAMPLE

Dilute donor's serum **1:100** in Serum Diluent.
Take 0.1 mL of donor serum and add to 10 mL of Serum Diluent.

REAGENT PREPARATION AND STORAGE

1. Wash Buffer:

Wash the contents of the vial into a 2000 mL flask with distilled water and Q.S. to 2000 mL mark with distilled water. Label it as Working Wash Buffer and store refrigerated at 2 °C - 8 °C.
The Working Wash Buffer is stable for 6 months at 2 °C - 8 °C.

2. Substrate Solution:

Mix Substrate Solution "A" and "B" in equal proportions 30 minutes before use.
(For example mix 5 mL each of "A" and "B" for each donor or plate to be used).
Discard the unused substrate mix solution. Do not interchange the caps on these solutions. If the mixed substrate solution looks blue in color before use, it should be discarded.
Mixed substrate solution is stable for 60 minutes at room temperature.

ASSAY PROCEDURE

Bring all the test kit reagents to room temperature before use.

1. PREPARATION OF CALIBRATION CURVE:
   Label four 12 x 75 mm glass tubes as 50, 100, 200 & 400 U/mL.
   Dispense 150 µL of Serum Diluent into these four tubes.
   Add 150 µL of Food Calibrator to the tube labeled 400 U/mL.
   Mix and transfer 150 µL into tube labeled 200 U/mL.
   Mix and transfer 150 µL into the tube labeled 100 U/mL.
   Again mix and transfer 150 µL into the tube labeled 50 U/mL.
   At this point you should have 150 µL in tubes 100, 200 & 400 U/mL, and 300 µL in tube 50 U/mL.
   This is the calibration curve to be used in the assay.
   Transfer 100 µL from each of these tubes to the microplate as follows:

Tube Label	Well Label
50 U/mL	1B
100 U/mL	1C
200 U/mL	1D
400 U/mL	1E

Add 100 µL of Serum Diluent to Well 1A and 100 µL of Positive Control to well 1F.

2. Place 100 µL of the diluted donor serum (**See Preparation of the Donor Sample – Section 6 above**) into all the other wells. There should be 100 µL of liquid in all the wells.
3. Cover the plates with parafilm or plastic wrap and incubate at room temperature (22 °C – 25 °C) for 1 hour.
4. After one-hour incubation, wash all the microwells three times with 300 µL of working wash buffer each time. (**See Reagent Preparation – Section 7**). If you use an automated washer, check the manufacturer's instructions for a three cycle wash procedure with 300 µL wash volume.
5. Add 100 µL of Food IgG-HRP Conjugate to all the wells.
6. Incubate the plates for 30 minutes at room temperature (22 °C – 25 °C).
7. Wash the plates again as in step #4.
8. Add 100 µL of Working Substrate mix to all the wells (see Reagent Preparation and Storage).
9. Cover the plates and Incubate for 10 minutes at room temperature (22 °C – 25 °C).
10. Add 50 µL of Stopping Solution to all the wells. (Blue color in the wells will change to yellow).
11. Set the microplate reader at 450 nm and read absorbance in all the wells.
12. Plot the curve, using absorbance vs. the concentrations in the wells 1A -1E, on linear graph paper. A sample curve is given below. **Do not use sample for data calculation**.

Well Position	Concentration	Absorbance
1A	0 U/mL	0.070
1B	50 U/mL	0.562
1C	100 U/mL	0.858
1D	200 U/mL	1.221
1E	400 U/mL	1.767
1F	Pos. Cont.	1.408
1G	Pat. Sample	0.280

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90 Foods (1 Person Sample) Microplate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	Apple	Butter	Cheddar Cheese	Cola nut	Garlic	Lettuce, Iceberg	Oat	Pinto bean	Sardine	Strawberry	Trout
B	Calibrator 1	Avocado	Cabbage	Chicken	Corn	Goat's Milk	Lemon	Olive	Pineapple	Scallop	String bean	Tuna
C	Calibrator 2	Banana	Cane sugar	Chili Pepper	Cottage Cheese	Grape White/concord,	Lima bean	Onion	Pork	Sesame	Sunflower seed	Turkey
D	Calibrator 3	Barley, whole grain	Cantaloupe	Chocolate	Cow's Milk	Grapefruit	Lobster	Orange	Potato	Shrimp	Sweet potato	Walnut, black
E	Calibrator 4	Beef	Carrot	Cinnamon	Crab	Green pea	Malt	Oyster	Rice	Sole	Swiss Cheese	Wheat
F	Positive Control	Blueberry	Cashew	Clam	Cucumber	Green pepper	Millet	Parsley	Rye	Soybean	Tea, black	Yeast, Baker's
G	Almond	Broccoli	Cauliflower	Codfish	Egg, white/yolk	Halibut	Mushroom	Peach	Safflower seed	Spinach	Tobacco	Yeast, Brewer's
H	American Cheese	Buckwheat	Celery	Coffee	Eggplant	Honey	Mustard Seed	Peanut	Salmon	Squashes	Tomato	Yogurt

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