DRG® Anti-Deaminated Gliadin Peptide IgG (EIA-5080)

USA: RUO

Revised 14 Oct. 2013 rm (Vers. 5.1)

This kit is intended for Research Use Only.

Not intended for use in diagnostic procedures.

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

1 INTENDED USE

The Anti-Deamidated Gliadin Peptide (DGP) IgG kit is an indirect solid phase enzyme assay (ELISA) kit designed for measurement of IgG class antibodies directed against Deamidated Gliadin Peptides (DGP) in human serum or plasma.

The Anti-Deamidated Gliadin Peptide (DGP) IgG kit is intended for laboratory use only.

2 PRINCIPLE

The Anti-Deamidated Gliadin Peptide (DGP) IgG test is based on the binding of present antibodies in calibrators, controls or prediluted samples on the synthetic deamidated gliadin peptides (DGP) coated on the inner surface of the wells. After a 30 minutes incubation the microplate is washed with wash buffer for removing non-reactive serum components.

An anti-human-IgG horseradish peroxidase conjugate solution recognizes IgG class antibodies bound to the immobilized antigens. After a 30 minutes incubation any excess enzyme conjugate, which is not specifically bound is washed away with wash buffer.

A chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation the color development is stopped by adding the stop solution. The solutions color changes into yellow. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

3 REAGENTS, MATERIALS AND INSTRUMENTATION

3.1 Reagents and materials supplied in the kit

1. Anti-DGP **Standards S0 – S4**; (5 vials, 1.2 mL each))
   Phosphate buffer 0.1 M, NaN_3 < 0.1%, human serum
2. **Control** (2 vials, 1.2 mL each, ready to use) ,
   Phosphate buffer 0.1 M, NaN_3 < 0.1%, human serum
   low and high control
3. **Sample Diluent** (1 vial, 100 mL)
   Phosphate buffer 0.1 M, NaN_3 < 0.1%
4. **Enzyme Conjugate** (1 vial, 15 mL)
   Anti h-IgG conjugated with horseradish peroxidise (HRP), BSA 0.1%, Proclin < 0.0015%
5. **DGPCoated Microplate** (1 microplate breakable)
6. **TMB-Substrate Solution** (1 vial, 15 mL)
   3,3',5,5'-tetramethylbenzidine 0.26 g/L, hydrogen peroxide 0.05%, Proclin < 0.0015%
7. **Stop Solution** (1 vial, = 15 mL)
   Sulphuric acid 0.15 M

8. **Wash Solution** 10X Concentrate (1 vial, 50 mL)
   Phosphate buffer 0.2 M, Proclin < 0.0015%

3.2 Reagents necessary not supplied

Distilled water.

3.3 Auxiliary materials and instrumentation

Automatic dispenser.

Microplate reader (450 nm)

4 WARNINGS

1. This kit is intended for Research Use by professional persons only. Not for internal or external use in Humans or Animals.
2. Use appropriate personal protective equipment while working with the reagents provided.
3. Follow Good Laboratory Practice (GLP) for handling blood products.
4. All human source material used in the preparation of reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Standard and the Controls should be handled in the same manner as potentially infectious material.
5. Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
6. Some reagents contain small amounts of Sodium Azide (NaN_3) or Proclin 300® as preservatives. Avoid the contact with skin or mucosa.
7. Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
8. The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
9. The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
10. Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5 PRECAUTION

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2 °C - 8 °C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22 °C - 28 °C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vial labels must be observed. Do not use any kit component beyond their expiry date.

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- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices**, before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips. For this purpose, DRG supplies a separate decontamination reagent for cleaning needles.
- If you use automated equipment , the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6 PROCEDURE

6.1 Preparation of the Standard (S0 – S4)

Since no international reference preparation for Anti-DGP antibodies is available, the assay system is calibrated in relative arbitrary units (AU). The standards are ready to use and have the following concentration:

	S0	S1	S2	S3	S4
AU/mL	0	15	30	60	240

Once opened, the Standards are stable 6 months at 2 °C - 8 °C.

6.2 Preparation of the Sample

For determination of Anti-DGP human serum or plasma are the preferred sample matrixes.

All serum and plasma samples have to be prediluted with sample diluent 1 : 100:
for example 10 µL of sample may be diluted with 990 µL of sample diluent.

The sample need not be taken from fasting individuals, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum (after clot formation) or plasma from the cells by centrifugation.

Samples may be stored refrigerated at 2 °C - 8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20 °C. To avoid repeated thawing and freezing the samples should be aliquoted.

Neither Bilirubin nor Hemolysis have significant effect on the procedure.

The Controls are ready to use.

6.3 Preparation of the Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use.
For smaller volumes respect the 1:10 dilution ratio.
The diluted wash solution is stable for 30 days at 2 °C - 8 °C.

In concentrated wash solution it is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

6.4 Procedure

Allow all reagents to reach room temperature (22 °C – 28 °C) for at least 30 minutes.

Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2 °C - 8 °C.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the standard curve (S0-S4), two for each Control, two for each sample, one for Blank.

Reagent	Standard	Sample or Controls	Blank
Standard S0-S4	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate 30 minutes at room temperature (22 °C – 28 °C). Remove the contents from each well, wash the wells 3 times with 300 µL diluted wash solution			
Conjugate	**100 µL**	**100 µL**	
Incubate 30 minutes at room temperature (22 °C – 28 °C). Remove the contents from each well, wash the wells 3 times with 300 µL of diluted wash solution.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate 15 minutes in the dark at room temperature (22 °C – 28 °C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank within 5 minutes.			

7 RESULTS

7.1 Standard Curve

For Anti-Deamidated Gliadin Peptide (DGP) IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable.

However we recommend using a Lin-Log curve.

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Typical Results (example only)

The figures below show typical results for Anti-Deamidated Gliadin Peptide (DGP) IgG. These data are intended for illustration only and should not be used to calculate results from another run.

N	OD1	OD2	Mean OD	Conc. 1	Conc. 2	Mean Conc.	CV %
S0	0.011	0.011	0.011	0.09	0.09	0.09	6E-7
S1	0.165	0.161	0.163	14.74	14.36	14.55	1.89
S2	0.322	0.324	0.323	30.46	30.67	30.57	0.48
S3	0.590	0.590	0.590	59.78	59.78	59.78	4E-7
S4	1.694	1.768	1.731	232.1	248.1	240.1	4.71

8 REFERENCE VALUES

In smaples from a group of healthy inviduals, the following rnages have been observed with the Anti-Gliadin tests:

	Anti-DGP-Ab IgG [AU/mL]
Low:	<15
Equivocal:	15-=30
High:	>30

Each laboratory should produce their own range of expected values based on the indigenous population where the laboratory works.

9 WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

10 BIBLIOGRAPHY

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11 TROUBLESHOOTING

ERRORS / POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

1. no conjugate pipetted reaction after addition
2. contamination of conjugates and/or of substrate
3. errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

4. incorrect conjugate (e.g. not from original kit)
5. incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

6. incorrect conjugate (e.g. not from original kit)
7. incubation time too long, incubation temperature too high
8. water quality for wash buffer insufficient (low grade of deionization)
9. insufficient washing (conjugates not properly removed)

Unexplainable outliers

10. contamination of pipettes, tips or containers
11. insufficient washing (conjugates not properly removed)

12. too high within-run CV%
13. reagents and/or strips not pre-warmed to room temperature prior to use
14. plate washer is not washing correctly (suggestion: clean washer head)
15. too high between-run CV %
16. incubation conditions not constant (time, temperature)
17. controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
18. person-related variation

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