Version 26-05/24

User's Manual

Hemoglobin/Haptoglobin-Complex ELISA Kit

REF **DEIA051J**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Hemoglobin/Haptoglobin Complex ELISA is intended for the quantitative determination of hemoglobin/haptoglobin complex in human stool samples. For Research Use Only. Not for use in diagnostic procedures.

Principles of Testing

This ELISA is used for quantitative determination of hemoglobin/haptoglobin complex in stool. The complex in the sample is bound to anti-haptoglobin antibodies (in excess), which are immobilized on the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, an anti-hemoglobin peroxidase-labeled antibody is added. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, tetramethylbenzidine (TMB). An acidic solution is then added to stop the reaction. The color converts from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of hemoglobin/haptoglobin complex in the sample. A standard curve is generated with the values obtained from the standard (optical density vs. concentration). The presence of hemoglobin/haptoglobin complex is determined directly from this curve.

Reagents And Materials Provided

1. Hemoglobin/Haptoglobin Microplate (96 wells), 12 strips of 8 wells, Ready to use
2. Standards (1-5) (0, 0.67, 3.3, 10, 50 µg/g), 2 x 5 vials, Lyophilized
3. Control Levels 1 and 2, 2 vials each, Lyophilized
4. Wash Buffer Concentrate, 2 x 100 mL, 10X
5. Extraction Buffer Concentrate, 1 x 100 mL, 2.5X
6. Conjugate, 1 x 15 mL, Ready to use
7. Sample Dilution Buffer, 2 x 15 mL, Ready to use
8. TMB Substrate, 15 mL, Ready to use
9. Stop Solution, 15 mL, Ready to use

Materials Required But Not Supplied

1. Precision pipettes for dispensing up to 1000 µL (with disposable tips)
2. Repeating or multi-channel pipette for dispensing up to 1000 µL
3. Volumetric containers and pipettes for reagent preparation
4. Distilled/Deionized water for reagent preparation
5. Microplate washer or wash bottle
6. Microplate shaker capable of 700-900 rpm
7. Microplate reader

8. Centrifuge (1,000 x g to 3,000 x g)
9. Vortex for sample preparation
10. Laboratory Balance
11. Foil to cover the microplate
12. Timer

Storage

The kit should be stored at 2-8°C. The kit is stable until the expiration date on the box label.

Specimen Collection And Preparation

Stool samples are appropriate for use in this assay.

Sample Stability and Storage

Raw Stool

Due to the degradation of hemoglobin/haptoglobin at room temperature, which can amount to 50% per day, raw stool samples should be shipped frozen. If shipment either at -20°C or cooled is not possible, the samples can be mailed overnight. However, this will reduce the sensitivity of the assay. Raw stool can be stored at -20°C for 1 month.

Stool Extract

Stool extract is stable at room temperature (15-30°C), 2–8°C, as well as at -20°C for seven days. Avoid more than three freeze-thaw cycles.

Extraction of the Stool Sample:

1X working extraction buffer is used as a sample extraction buffer. It is recommended to perform the following sample preparation:

1. The raw stool sample has to be thawed. For particularly heterogeneous samples it is recommended to perform a mechanical homogenization using an applicator, inoculation loop or similar device.
2. Fill the empty sample tube with 1.5mL of 1X working extraction buffer (diluted 1:2.5) before using it with the sample. Important: Allow the extraction buffer to reach room temperature. If using the EZ Extraction Device, the device is provided in a prefilled format and does not require any additional extraction buffer.
3. If using the device, unscrew the tube (yellow part of cap) to open. Insert yellow dipstick into sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off and leave 15 mg of sample to be diluted. Screw tightly to close the tube.
4. Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for approximately 10 minutes improves the result.
5. Allow sample to stand for approximately 10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
6. Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again. This will result in a dilution of 1:100

(Dilution I).

The sample suspension is now ready for use.

Plate Preparation

Below is a suggested plate layout for running standards, controls, and up to 41 samples in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	2	2	10	10	18	18	26	26	34	34
B	Std 2	Std 2	3	3	11	11	19	19	27	27	35	35
C	Std 3	Std 3	4	4	12	12	20	20	28	28	36	36
D	Std 4	Std 4	5	5	13	13	21	21	29	29	37	37
E	Std 5	Std 5	6	6	14	14	22	22	30	30	38	38
F	Ctrl 1	Ctrl 1	7	7	15	15	23	23	31	31	39	39
G	Ctrl 2	Ctrl 2	8	8	16	16	24	24	32	32	40	40
H	1	1	9	9	17	17	25	25	33	33	41	41

Std = Standard

Ctrl = Control

Numbered wells = Samples

Reagent Preparation

All reagents must be equilibrated to room temperature prior to preparation and subsequent use in the assay. Prepare enough reagents for only the number of strips used. The kit can be used up to 4 times within the expiry date stated on the label. Reagents with a volume less than 100 µL should be centrifuged before use to avoid loss of volume.

Standards and Controls (Levels 1 and 2) are provided in a lyophilized form and are stable at 2-8°C until the expiry date stated on the label. Before use, reconstitute in 500 µL of distilled water and mix by gently inverting to ensure completed reconstitution. Allow the vial contents to dissolve for 10 minutes and then mix thoroughly. Reconstituted standards and controls can be stored at 2–8°C for 4 weeks.

Wash Buffer Concentrate (10X) must be diluted with distilled or deionized water 1:10 before use (100mL WASHBUF + 900mL distilled water) and mixed well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be dissolved at room temperature or in a water bath at 37°C before dilution of the buffer solutions. The WASHBUF is stable at 2–8°C until the expiry date stated on the label. 1X working Wash buffer (1:10 diluted WASHBUF) can be stored in a closed flask at 2–8°C for one month.

Extraction Buffer Concentrate (2.5X) must be diluted with distilled or deionized water 1:2.5 before use (100mL Extraction Buffer + 150mL distilled water), mix well. Crystals could occur due to high salt concentration in the stock solutions. Before dilution, the crystals must be dissolved at 37°C in a water bath. The Extraction Buffer is stable at 2–8°C until the expiry date stated on the label. 1X working extraction buffer (1:2.5 diluted) can be stored in a closed flask at 2–8°C for up to four months.

All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at 2–8°C.

Assay Procedure

All reagents and microplate strips (while sealed in foil pouch) should be equilibrated to room temperature prior to use. Gently mix all reagents before use. A standard curve must be performed with each assay run and with each microplate if more than one is used at a time. All standards, controls, and samples should be run in duplicate.

For automated ELISA processors the given protocol may need to be adjusted according to the specific features of the respective automated platform.

1. Bring all reagents and samples to room temperature (15–30°C) and mix well.
2. Before use, wash wells 5 times with 250 µL 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
3. Add 50 µL of Sample Dilution Buffer to each well.
4. Add 50 µL of prepared standards, controls, and samples into respective wells. See Reagent Preparation for control reconstitution instructions. A suggested plate layout is included.
5. Cover the strips and incubate for 1 hour at room temperature (15–30°C).
6. Discard the contents of each well. Wash each well 5 times with 250 µL of 1X working wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
7. Add 100 µL of conjugate into each well.
8. Cover the strips and incubate for 1 hour at room temperature (15–30°C).
9. Discard the contents of each well. Wash each well 5 times with 250 µL of wash buffer. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
10. Add 100 µL of substrate into each well.
11. Incubate for 10–20 minutes at room temperature (15–30°C) in the dark*
12. Add 100 µL of stop solution into each well, mix thoroughly.
13. Determine absorption immediately with an ELISA reader at 450nm against 620nm (or 690nm) as a reference. If no reference wavelength is available, read only at 450nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405nm against 620nm as a reference.

* The intensity of the color change is temperature sensitive. It is recommended to observe the color change and to stop the reaction upon good differentiation.

Quality Control

Control samples should be analyzed with each run. Results generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Calculation

The following algorithms can be used alternatively to calculate the results. It is recommended to use the 4-parameter algorithm.

1. 4-parameter algorithm: It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with

a value less than 1 (e.g. 0.001).

2. Point-to-point calculation: It is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.
3. Spline algorithm: it is recommended to use a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the program used, a comparison of the paired values should be done manually.

Stool Samples

Since the sample dilution is already considered in the calibration curve, the dilution factor is 1. In case another dilution factor has been used, multiply the obtained result with the dilution factor used.

Precision

Repeatability (Intra-Assay)

The repeatability was assessed with 2 stool samples under constant parameters (same operator, measurement system, day and kit lot).

	Sample 1	Sample 2
Mean	8.31 µg/g	3.88 µg/g
CV (%)	3.9	2.5
n	42	42

Reproducibility (Inter-Assay)

The reproducibility was assessed with 2 control samples under varying parameters (different operators, measurement systems, days, and kit lots).

	Sample 1	Sample 2
Mean	1.16 µg/g	4.20 µg/g
CV (%)	4.5	2.7
n	30	30

Sensitivity

Analytical Sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors:

Limit of Blank, (LoB) 0.033 µg/g

Limit of Detection (LoD) 0.158 µg/g

Limit of Quantitation (LoQ) 0.248 µg/g

The specified accuracy goal for the LoQ was 20% CV.

Precautions

1. The human blood products incorporated into this kit have been tested for the presence of HIV (Human Immunodeficiency Virus), HBV (Hepatitis B Virus), and HCV (Hepatitis C Virus). Test methods for these viruses do not guarantee the absence of a virus; therefore, all reagents should be treated as potentially infectious. Handling and disposal should be in accordance with all appropriate national and local regulations for the handling of potentially biohazardous materials.
2. Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are hazardous to health and the environment. Substrates for enzymatic color reactions may also cause skin and/or respiratory irritation. Avoid contact with the substances must be avoided.
3. The 10X wash Buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of contact. Warning: Causes serious eye irritation. If In Eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists, get medical advice / attention.
4. The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.
5. Avoid direct contact with skin.
6. This product is not for internal use.
7. Avoid eating, drinking, or smoking when using this product.
8. Do not pipette any reagents by mouth.
9. Reagents from this kit are lot-specific and must not be substituted.
10. Do not use reagents beyond the expiration date.
11. Variations to the test procedure are not recommended and may influence the test results.

Technical Hints

1. Do not interchange different lot numbers of any kit component within the same assay. Furthermore, it is not recommended to assemble the wells of different microtiter plates for analysis, even if they are of the same batch as wells from already opened microtiter plates are exposed to different conditions than sealed ones.
2. Control samples should be analyzed with each run.
3. Reagents should not be used beyond the expiration date stated on kit label.
4. Substrate solution should remain colorless until use.
5. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
6. Avoid foaming when mixing reagents.
7. Do not mix plugs and caps from different reagents.
8. The assay should always be performed according to the enclosed manual.

General Notes on the Test and the Test Procedure

1. Control samples should be analyzed with each run.
2. The guidelines for medical laboratories should be followed.
3. Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the

results of the test.

Limitations

Samples with concentrations above the measurement range can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used.

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used.