Epitope Diagnostics, Inc.

q-FOB™ Quantitative Fecal Occult Blood Test Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative Measurement of Human Hemoglobin Level in Stool

REF **KT 850** Σ **12x8** **2-8°C**

EU: IVD CE

For In Vitro Diagnostic Use

INTENDED USE

This q-FOB™ test kit is intended for detection of fecal occult blood by the quantitative determination of human hemoglobin levels in stool samples. This assay exclusively measures human hemoglobin without cross reaction to animal blood. The test is useful for detecting the severity of gastrointestinal bleeding and in the aid of screening for colorectal adenoma/polyps and cancer, as well as other inflammatory bowel diseases (Crohn's disease, Ulcerative Colitis, etc.).

INTRODUCTION

Detection of abnormally high level of fecal hemoglobin is recommended by America Cancer Society (ACS), Center for Disease Control and Prevention (CDC) and Center for Medical Service (CMS) of the Department of Health of the United States. The Fecal Occult Blood (FOB) Rapid Test was used in the past 40 years. Screening for occult blood by means of guaiac tests has an unsatisfactory sensitivity for the detection of colorectal neoplasm with a dietary restriction draw back. Immunochemical FOB test dramatically increases the analytical sensitivity and specificity in the detection of human hemoglobin in feces. A few clinical trials showed that immunochemical FOB test is superior in clinical diagnostic sensitivity and specificity compared to guaiac FOB test. However, these rapid tests do not give an insight to the severity of the bleeding in the lower gastrointestinal system.

This q-FOB™ assay using human hemoglobin specific monoclonal antibodies would bring significant advantages over the qualitative rapid FOB tests. The assay does not require dietary restrictions such as no raw meat, vitamin C rich food (salad, fruits, etc.). This q-FOB™ assay detects human hemoglobin level in 100-fold lower concentrations than the guaiac FOB test and avoids false-negative results. Because highly specific human hemoglobin monoclonal antibodies are used, false positive results are practically excluded.

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human hemoglobin in stool sample. The assay utilizes the two-site "sandwich" technique with two selected antibodies that bind to different epitopes of human hemoglobin.

Assay standards, controls and patient samples are added directly to wells of a microtiter plate that is coated with streptavidin. Subsequently, a mixture of biotinylated human hemoglobin specific monoclonal antibody and a horseradish peroxidase (HRP) conjugated human hemoglobin specific monoclonal antibody is added to each well. After the first incubation period, a "sandwich" of "biotinylated monoclonal antibody - human hemoglobin – HRP conjugated monoclonal antibody" is formed and this immunocomplex is also captured to the wall of microtiter plate via a streptavidin-biotin affinity binding. The unbound monoclonal antibodies and buffer matrix is removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human hemoglobin in a test sample. A standard curve is generated by plotting the absorbance versus the respective human hemoglobin concentration for each standard on point-to-point or 4 parameter curve fitting. The concentration of fecal human hemoglobin in test samples is determined directly from this standard curve.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

1. **Streptavidin Coated Microplate (Cat. No. 10040)**
One microplate with 12 x eight strips (96 wells total) coated with streptavidin. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **Hemoglobin Tracer Antibody (Cat. No. 30219)**
One vial containing 0.9 mL HRP labeled anti-human hemoglobin antibody in a stabilized protein matrix. This reagent must be diluted with Hemoglobin Capture Antibody (Cat# 30220) before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **Hemoglobin Capture Antibody (30220)**
Two vials, each contains 9 mL biotinylated anti-human hemoglobin capture antibody in a stabilized protein matrix. This reagent must be added with Hemoglobin Tracer Antibody (Cat# 30219) before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **ELISA Wash Concentrate (Cat. No. 10010)**
One bottle contains 30 mL of 30 fold concentrate. Before use the contents must be diluted with **870 mL** of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

5. **ELISA HRP Substrate (Cat. No. 10020)**
One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

6. ELISA Stop Solution (Cat. No. 10030)
One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent may be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

7. Human Hemoglobin Standards (Cat. No. 30221 – 30225)
One vial contains liquid standard level 1. Four vials each contain human hemoglobin in a lyophilized bovine serum based matrix with a non-azide, non-mercury preservative. **Refer to vials for exact concentration for each standard.** These reagents should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

8. Human Hemoglobin Controls (Cat. No. 30226 – 30227)
Two vials each contains human hemoglobin in a lyophilized bovine serum based matrix with a non-azide, non-mercury preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8°C and are stable until the expiration date on the kit box.

SAFTY PRECAUTIONS

The reagents must be used in professional laboratory. Source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potential infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause sever irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. **Fecal sample collection tube (Epitope Catalog No: 30210)**
2. Precision single channel pipettes capable of delivering 25 µL, 100 µL, and 1000 µL etc.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable plastic 100 mL and 1000 mL bottle with caps.
5. Aluminum foil.
6. Deionized or distilled water.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION

Only one fecal sample is required. Fresh fecal sample must be collected by using Epitope Diagnostics Fecal Sample Collection Device (Cat# 30210). This tube is specially designed for easy collection of a substantially small amount of fecal sample into the tube pre-filled with sample extraction buffer. The collected fecal sample must be transported, kept at 2-8°C and tested within 3 days. Otherwise**, fecal sample must be stored below -20°C for a longer storage period**. Avoid more than three times freeze- thaw cycle for each specimen.

It is strongly recommended to use Epitope Diagnostics Fecal Sample Collection Device (Cat# 30210) for sample collection. The clinical validation data of this test were generated by using this sampling tube!

ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.

(3) Reconstitute all assay standard level 2 to level 5 and controls by adding **0.5 mL** of demineralized water to each vial. Allow the standards and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. One must make sure that all solid is dissolved completely prior to use. These reconstituted standards and controls must be stored at - 10°C or below after assay. Do not exceed 3 freeze-thaw cycles.

(4) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	SAMPLE 3
B	STD 1	STD 5	SAMPLE 4
C	STD 2	C 1	SAMPLE 5
D	STD 2	C 1	SAMPLE 6
E	STD 3	C 2	SAMPLE 7
F	STD 3	C 2	SAMPLE 8
G	STD 4	SAMPLE 1	
H	STD 4	SAMPLE 2	

(5) Place a sufficient number of streptavidin coated microwell strips in a holder to run human hemoglobin standards, controls and unknown samples in duplicate.

(6) Prepare working Tracer Antibody and Capture Antibody mixture by 1:21 fold dilution of the Hemoglobin Tracer Antibody through adding the tracer antibody into the capture antibody. Following is a table that outlines the relationship of strips used and antibody mixture prepared.

Strip no.	Capture Antibody	Tracer Antibody
1	2 mL	100 µL
2	3 mL	150 µL
3	4 mL	200 µL
4	5 mL	250 µL
5	6 mL	300 µL
6	7 mL	350 µL
7	8 mL	400 µL
8	9 mL	450 µL
9	10 mL	500 µL
10	11 mL	550 µL
11	12 mL	600 µL
12	13 mL	650 µL

Note: this antibody mixture should be freshly prepared before running the assay.

(7) Load all reagents onto a DS2 or DSX system according to the computer program.

2. Patient Sample Preparation

If the Epitope Diagnostics Fecal Sample Collection Tube (Cat# 30210) is use, there is no sample preparation being required.

Before assay bring all patient samples to room temperature. Unscrew the **natural cap** of the sample collection tube and load the samples onto the DS2 or DSX system. It is important to make sure that there is not substantially amount of air bubbles on the surface of the collection tube. It is preferred to gently vortex the tube before loading onto the system.

3. Assay Procedure
This assay procedure is programmed for the DS2 or DSX system.

(1) Add **25 µL** of standards, controls and patient samples into the designated microwell.
(2) Add **100 µL** of above antibody mixture to each well
(3) Incubate plate at 37°C, shaking for **20 minutes**.
(4) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(5) Add **100 µL** of ELISA HRP Substrate into each of the wells.
(6) Incubate plate at 37°C, shaking for **8 minutes**.
(7) Add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.
(8) Read the absorbance at 450 nm.

Note: an alternative manual test can be performed by using the assay procedure as described below.

(1) Add **25 µL** of standards, controls and patient samples into the designated microwell.
(2) Add **100 µL** of above antibody mixture to each well
(3) Mix gently and cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light. Incubate plate at room temperature for **40 minutes**.
(4) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(5) Add **100 µL** of ELISA HRP Substrate into each of the wells.
(6) Cover the plate with an aluminum foil to avoid exposure to light. Incubate plate at room temperature for **20 minutes**.
(7) Add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.
(8) Read the absorbance at 450 nm.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused streptavidin coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETATION OF RESULTS

For the DS2 and DSX system, it is recommended to use a linear/linear, point-to-point or 4-parameter standard curve fitting.

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the Diluted Fecal Sample Extraction Buffer (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

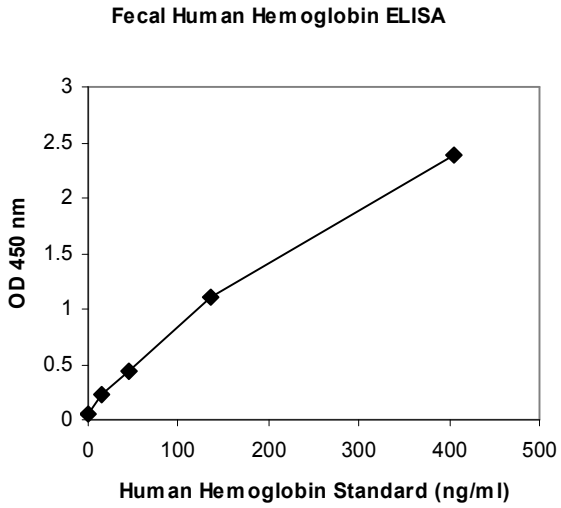
The fecal human hemoglobin concentrations for the controls and the patient samples are read directly from the standard curve using their respective corrected absorbance.

Patient Fecal Hemoglobin (µg Hemoglobin/gram stool) = Read value directly from assay (ng/ml) x 0.5

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this fecal human hemoglobin ELISA are represented. **This curve should not be used in lieu of standard curve run with each assay.**

Well I.D.	OD 450 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.056 0.059	0.058	0.000	
15 ng/mL	0.215 0.225	0.220	0.162	
45 ng/mL	0.466 0.425	0.445	0.387	
135 ng/mL	1.096 1.122	1.109	1.051	
405 ng/mL	2.285 2.483	2.384	2.326	
Control 1	0.271 0.270	0.271	0.213	21.71 ng/mL
Control 2	0.889 0.910	0.900	0.842	106.60 ng/mL



EXPECTED VALUES

Stool samples from normal healthy adults with age of 17 – 74 were collected and measured with this ELISA. Following is a recommended cut-off for patient sample interrelation.

	Fecal Hemoglobin (ng/ml)	Fecal Hemoglobin (µg Hb / g stool)
Negative (normal)	< 20	< 10
Positive (light)	20 - 50	10 - 25
Positive (medium)	50 - 200	25 - 100
Positive (strong)	> 200	> 100

Each laboratory has the choice to use either nano-gram hemoglobin per milliliter extraction buffer or micro-gram hemoglobin per gram stool for report the test results.

LIMITATION OF THE PROCEDURE

1. A strong positive of fecal hemoglobin is likely to indicate a more significant clinical pathological condition of a patient. However, light positive of fecal hemoglobin does not indicate a lesser possibility of polyps, adenoma or cancer.
2. A normal fecal hemoglobin level does not rule out the presence of any gastrointestinal diseases.
3. For sample values reading greater than highest standard, it is recommend to re-assay samples with dilution.
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the human hemoglobin ELISA as determined by the 95% confidence limit on 20 duplicate determination of zero standard is approximately 0.5 ng/mL.

High Dose "hook" effect

This assay has showed that it did not have any high dose "hook" for fecal sample hemoglobin level up to 2,000,000 µg/gram stool.

Precision

The intra-assay precision is validated by measuring two controls samples in a single assay with 16-replicate determinations.

Mean Hemoglobin Value (ng/mL)	CV (%)
24.46	7.6
140.39	9.1

The inter-assay precision is validated by measuring two control samples in duplicate in 16 individual assays.

Mean Chromogranin A Value (ng/mL)	CV (%)
20.66	6.9
102.47	8.0

The inter-sample precision was performed by collecting two specimens from one bowel movement. These paired samples are measured in an assay according to the assay procedure. The results indicate that there are very satisfactory agreements of the two samples collected from one bowel movement.

	Fecal Hemoglobin (ng/ml)		
Patients	Sample 1	Sample 2	CV%
1	9.3	7.5	15
2	1.6	1.2	20
3	22.5	20.2	7.6
4	482.3	460.5	3.3
5	869.7	872.1	0.2
6	726.4	764.7	3.6

Linearity

Two samples were diluted with assay buffer and assayed. The results in the value of ng/mL are as follows:

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Neat	410.0	-	-
	1:2	235.5	205	115
	1:4	105.4	102.5	103
	1:8	47.7	51.2	93
2	Neat	191.8	-	-
	1:2	89.2	95.9	93
	1:4	47.3	48.0	99
	1:8	17.7	24.0	74

Recovery

Four assay standards were spiked together in equal volume (1 vol. + 1 vol. mixture) and assayed. The results in the value of ng/mL are as follows:

#	Orig. Value	Amount Spiked	Observed Value	Expected Value	Recovery %
1	15	45	32.3	30.0	108
2	45	135	97.4	90.0	108
3	135	405	278.0	270	103

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.
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Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
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In Vitro Diagnostic Device	Use by
Read instructions before use	Lot No.
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