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 CE IVD 96

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.).This kit is for in vitro diagnostic use only.

SUMMARY OF PHYSIOLOGY

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 drawback. 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 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 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 hemoglobin antibodies. Subsequently, a horseradish peroxidase (HRP)-conjugated human hemoglobin specific antibody is added to each well. After an incubation period, a "sandwich" of "antibody – human hemoglobin – HRP-conjugated antibody" is formed. The unbound antibody 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. Reagents from different kit lot numbers should not be combined or interchanged.

1. q-FOB Antibody Coated Microplate (30520)

Microplate coated with anti-hemoglobin.

Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use

2. q-FOB Tracer Antibody (30556)

HRP-labeled anti-human hemoglobin antibody in a stabilized protein matrix.

Qty: 1 x 0.9 mL
Storage: 2 – 8°C
Preparation: 21X Concentrate. The contents must be diluted with tracer antibody diluent (30525) and mixed well before use.

3. Tracer Antibody Diluent (30525)

Buffer for tracer antibody dilution according to the assay procedures.

Qty: 1 x 20 mL
Storage: 2 – 8°C
Preparation: Ready to Use

4. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL
Storage: 2 – 8°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

5. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use

6. ELISA Stop Solution (10030)

0.5 M sulfuric acid

Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use

7. q-FOB Calibrators Levels 1 to 5 (30561-30565)

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

Qty: 5 x Vials

Storage: 2 – 8°C , <-20°C for long term storage
Do not exceed 3 freeze-thaw cycles.

Preparation: Must be reconstituted with 1.0 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

8. q-FOB Controls (30566, 30567)

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

Qty: 2 x Vials

Storage: 2 – 8°C , <-20°C for long term storage
Do not exceed 3 freeze-thaw cycles.

Preparation: Must be reconstituted with 1.0 mL of demineralized water, allowed to sit for 10 minutes, and then mix microwell by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic use only. Source material which contains reagents of bovine serum albumin 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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. q-FOB™ Fecal Sample Collection Kit (30210)
2. Precision single channel pipettes capable of delivering 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 and 620 nm.

SPECIMEN COLLECTION & STORAGE

Only one fecal sample is required. Fresh fecal sample must be collected by using Epitope Diagnostics q-FOB™ Fecal Sample Collection Kit (30210). This tube is specially designed for easy collection of a substantially consistent 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 freeze- thaw cycles for each specimen.

It is strongly recommended to use Epitope Diagnostics q-FOB™ Fecal Sample Collection Kit (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 (20-25 ºC). Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate (10010) must be diluted to working solution prior use. Please see REAGENTS section for details.
3. Reconstitute all assay calibrators level 1 to level 5 (30561-30565) and controls (30566, 30567) by adding 1.0 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 may be stored at 2 – 8°C for up to 3 days or at –20°C or below for long-term storage. Do not exceed 3 freeze-thaw cycles.

2. Specimen Preparation

1. If the Epitope Diagnostics q-FOB™ Fecal Sample Collection Kit (Cat. 30210) is used, there is no sample preparation required.
2. For automated assays, bring all patient samples to room temperature. Unscrew the white cap of the sample collection tube and load the samples onto the DS2 or DSX system. It is important to make sure that there are no air bubbles on the surface of the collection tube.

3. Manual Assay Procedure

1. Place a sufficient number of q-FOB Antibody Coated microwell strips (30520) in a holder to run calibrators (30561-30565), controls (30566, 30567), and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
C	Calibrator Level 2	Control 1	SAMPLE 3
D	Calibrator Level 2	Control 1	SAMPLE 3
E	Calibrator Level 3	Control 2	SAMPLE 4
F	Calibrator Level 3	Control 2	SAMPLE 4
G	Calibrator Level 4	SAMPLE 1	SAMPLE 5
H	Calibrator Level 4	SAMPLE 1	SAMPLE 5

3. Add **100 µL** of calibrators (30561 - 30565), controls (30566, 30567), and samples into the designated microwells. Mix by gently tapping the plate.
4. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **60 minutes.**
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
6. Prepare the antibody working solution by 1:21 fold dilution of the tracer antibody (30556) with the diluent (30525). For each strip, it is required to mix 1 mL of the tracer antibody diluent with 50 µL of the tracer antibody in a clean test tube. *Note: This antibody working solution should be freshly prepared.*

7. Add **100 µL** of *antibody working solution* to each well. Mix by gently tapping the plate.
8. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **45 minutes.**
9. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
10. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells. Mix by gently tapping the plate.
11. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **15 minutes.**
12. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.
13. Read the absorbance at **450/620 nm** within **10 minutes** with a microplate reader.

4. Automated Assay Procedure

1. Add **100 µL** of standards, controls and patient samples into the designated microwell.
2. Incubate plate at **39 °C**, for **20 minutes** with initial shaking for two minutes at low speed.
3. Wash each well **5** times by dispensing **350 µL** of diluted wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
4. Add **100 µL** diluted Tracer Antibody to each of the wells.
5. Incubate plate at **39 °C**, for **12 minutes** with initial shaking for two minutes at low speed.
6. Wash each well 5 times by dispensing **350 µL** of diluted wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
7. Add **100 µL** of ELISA HRP Substrate (10020) into each of the wells.
8. Incubate plate at **39 °C** for **8 minutes** with initial shaking for 8 seconds at low speed.
9. Add **100 µL** of ELISA Stop Solution (10030) into each of the wells.
10. Read the absorbance at **450 nm** with a reference of **620 nm** and initial shaking for 3 seconds at low speed.

Note: Open automated ELISA system other than DS-2 can also be used. This may require adjustment of the protocol. It is very important to incubate the assay 18-22°C. A change of incubation temperature would cause unsatisfactory standard curve and erroneous test results.

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 antibody-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.

INTERPRETION OF RESULTS

1. For the DS2 and DSX system, it is recommended to use a linear/linear, point-to-point or 4-parameter standard curve fitting.
2. Calculate the average absorbance for each pair of duplicate test results.
3. 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.
4. 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.
5. 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

LIMITATIONS 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.

EXPECTED VALUES

Stool samples from normal healthy adults aged 17 – 81 were collected and measured with this ELISA. Following is a recommended cut-off for patient sample interrelation.

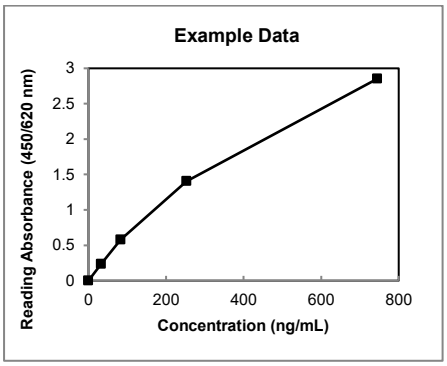
	Fecal Hemoglobin (ng/mL)	Fecal Hemoglobin (µg Hb /g stool)
Negative (normal)	< 58	< 29
Positive (light)	58 – 70	29 – 35
Positive (medium)	70 – 128	35 – 64
Positive (strong)	> 128	> 64

EXAMPLE DATA

A typical absorbance data and the resulting standard curve are represented.

Note: This curve should not be used in lieu of calibrator curve run with each assay.

Well ID	Reading Absorbance (450/620 nm)			Concentration (ng/mL)
	Readings	Average	Corrected	
Calibrator Level 1: 0 ng/mL	0.041	0.039	0.000	
	0.038			
Calibrator Level 2: 33 ng/mL	0.280	0.277	0.238	
	0.274			
Calibrator Level 3: 83 ng/mL	0.615	0.619	0.580	
	0.623			
Calibrator Level 4: 253 ng/mL	1.464	1.444	1.405	
	1.424			
Calibrator Level 5: 745 ng/mL	2.976	2.892	2.853	
	2.807			
Control 1	0.447	0.450	0.411	58.2
	0.452			
Control 2	1.054	1.054	1.016	173
	1.053			



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.

Reproducibility and Precision

The intra-assay precision is validated by measuring two controls samples in a single assay with 16-replicate determinations. The inter-assay precision is validated by measuring two control samples in duplicate in 16 individual assays. The results are as follows:

	Intra-Assay		Inter-Assay	
Sample	**1**	**2**	**1**	**2**
Mean (ng/mL)	60.7	90.2	68.3	206
CV (%)	3.8	3.1	6.9	6.6

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 is satisfactory agreement 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	461	3.3
5	870	872	0.2

Linearity

Two samples were diluted with assay buffer and assayed. The results are as follows:

Samples	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Sample A	410	-	-
50%	236	205	115
25%	105	103	103
12.5%	47.7	51.2	93
Sample B	192	-	-
50%	89.0	95.9	93
25%	47.3	48.0	99
12.5%	17.7	24.0	74

Recovery

Two assay calibrators were spiked together and assayed. The results are as follows:

Samples	Observed (ng/mL)	Expected Value (ng/mL)	Recovery (%)
Calibrator A	31.6	-	-
27.4	61.2	59.0	104
Calibrator B	22.4	-	-
91.5	98.9	114	108

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

1. Sieg A, Scheida M, John MR, Hertel A, Schroter M, Luthgens K, Schmidt-Gayk H. Validity of new immunological human fecal hemoglobin and albumin tests in detecting colorectal neoplasms-an endoscopy-controlled study. Z Gastroenterol. 1998 Jun;36(6):485-90. Review.
2. Sieg A, Hertel A, John MR, Luthgens K, Schmidt-Gayk H. Screening for colorectal neoplasms with a new immunological human faecal haemoglobin and albumin test. Eur J Cancer Prev. 1998 Aug;7(4):279-85.
3. Levi Z, Rozen P, Hazazi R, Vilkin A, Waked A, Maoz E, Birkenfeld S, Leshno M, Niv Y. A quantitative immunochemical fecal occult blood test for colorectal neoplasia. Ann Intern Med. 2007 Feb 20;146(4):244-55.
4. Fraser CG, Matthew CM, Mowat NA, Wilson JA, Carey FA, Steele RJ. Immunochemical testing of individuals positive for guaiac faecal occult blood test in a screening programme for colorectal cancer: an observational study. Lancet Oncol. 2006 Feb;7(2):127-31.
5. Federici A, Giorgi Rossi P, Borgia P, Bartolozzi F, Farchi S, Gausticchi G. The immunochemical faecal occult blood test leads to higher compliance than the guaiac for colorectal cancer screening programmes: a cluster randomized controlled trial. J Med Screen. 2005;12(2):83-8

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.

This product is developed and manufactured by

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

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GLOSSARY OF SYMBOLS (EN 980/ISO 15223)

IVD In Vitro Diagnostic Device	RUO For Research Use Only	LOT Lot Number
REF Catalog Number	Read instructions before use	Number of Tests
Store at	Use by	Keep away from heat and direct sun light
Manufacturer	EC REP Authorized Representative in Europe	CE European Conformity

SHORT ASSAY PROCEDURE

1. Manual Assay Procedure

1. Add **100 µL** of the calibrators, controls, and samples into the designated microwells.
2. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
3. Wash each well five times.
4. Add **100 µL** of the working tracer antibody to each well.
5. Cover and incubate at **room temperature (20-25 ºC)** for **45 minutes**.
6. Wash each well five times
7. Add **100 µL** of substrate to each well.
8. Cover and incubate at **room temperature (20-25 ºC)** for **15 minutes**.
9. Add **100 µL** of the stop solution to each well.
10. Read the absorbance at **450/620 nm**.

2. Automated Assay Procedure

1. Add **100 µL** of standards, controls and patient samples into the designated microwells.
2. Incubate plate at **39 °C**, for **20 minutes** with initial shaking for two minutes at low speed.
3. Wash each well **5** .
4. Add **100 µL** diluted Tracer Antibody to each of the wells.
5. Incubate plate at **39 °C**, for **12 minutes** with initial shaking for two minutes at low speed.
6. Wash each well 5 times.
7. Add **100 µL** of ELISA HRP Substrate into each of the wells.
8. Incubate plate at **39 °C** for **8 minutes** with initial shaking for 8 seconds at low speed.
9. Add **100 µL** of ELISA Stop Solution into each of the wells.
10. Read the absorbance at **450 nm** with a reference of **620 nm** and initial shaking for 3 seconds at low speed.