EPITOPE DIAGNOSTICS, INC.

EDI™ Quantitative Fecal Occult Blood (q-FOB) CLIA Kit

Chemiluminescence Immunoassay (CLIA) for the quantitative measurement of Human Hemoglobin in Feces.

REF CL0850 CE IVD 100, 150, 250 +2 +8 °C

INTENDED USE

This Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of human hemoglobin levels in fecesusing the ECL100 or ECL25 Immunoassay analyzer. This assay exclusively measures human hemoglobin without cross-reaction to animal blood. It is for in-vitro diagnostics use only.

SUMMARY OF PHYSIOLOGY

Fecal occult blood (FOB) refers to blood in feces that is not visibly apparent[3]. Presence of FOB is an indicator of gastrointestinal bleeding anywhere from the mouth to the colon[2]. While there are other technologies for detection of FOB, including rapid tests and guaiac, immunochemical tests have the best sensitivity and specificity[4,5,6,7]. Additionally, immunochemical tests utilize human hemoglobin-specific antibodies that detect lower concentrations, practically exclude false positives, and do not require dietary restrictions for sample collection[7,8]. Clinical applications may include screening for peptic ulcers[3], colorectal or gastric malignancy [5,6], and other inflammatory bowel diseases[1].

ASSAY PRINCIPLE

This CLIA is designed, developed, and produced for the quantitative measurement of q-FOB level in fecal samples. The assay utilizes a two-site “sandwich” technique with two antibodies that bind to different epitopes of hemoglobin.

Assay calibrators, controls, or patient samples are added directly to a reaction vessel containing streptavidin coated magnetic particles. Simultaneously, anacridinium ester antibody and a biotin antibody are added. The magnetic particles capture the biotin antibody as well as an immuno complex in the form of “magnetic particles – biotin hemoglobin antibody –hemoglobin – acridinium ester hemoglobin antibody”.

The materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the trigger solution is added to the reaction vessel and light generated by the reaction is measured with the ECL100 or ECL25 analyzer. The relative light units (RLU) are proportional to the concentration of hemoglobin in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve and reported in serum hemoglobin concentration.

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.

1. q-FOB Magnetic Particle Solution (L0401)

Qty:	1 x 2.8 mL (100/kit), 2 x 2.5 mL (150/kit), 3 x 2.5 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

2. Biotin q-FOB Antibody (L0402)

Qty:	1 x 6 mL (100/kit), 1 x 9 mL (150/kit), 1 x 14 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

3. Acridinium Ester q-FOB Antibody (L0403)

Qty:	1 x 11 mL (100/kit), 1 x 16.5 mL (150/kit), 1 x 26.5 mL (250/kit)
Storage:	2 – 8°C
Preparation:	Ready to Use

4. q-FOB Calibrators (L0404,L0407)

Lyophilized human hemoglobin in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty:	2 x vials
Storage:	2 – 8°C before reconstitution, <-20°C after reconstitution; Do not exceed 6 freeze-thaw cycles.
Preparation:	Must be reconstituted with 1.0 mL of demineralized water, allowed to sit for 10 minutes, and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

5. q-FOB Controls (L0408 – L0409)

Lyophilized human hemoglobin in a bovine serum albumin-based matrix with a non-azide preservative. Refer to vials for exact concentration.

Qty:	2 x vials
Storage:	2 – 8°C before reconstitution, <-20°C after reconstitution; Do not exceed 6 freeze-thaw cycles.
Preparation:	Must be reconstituted with 1.0 mL of demineralized water, allowed to sit for 10 minutes, and then mixed by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use.

SAFETY PRECAUTIONS

The reagents must be used in a professional laboratory environment and are for in vitro diagnostic use. 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. 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

The instrument only uses materials supplied by Epitope Diagnostics, Inc. When materials available from third-party

suppliers are used, Epitope Diagnostics, Inc. takes no responsibility for the validity of results obtained. Material is available for purchase from Epitope Diagnostics, Inc. Please contact your distributor for more information.

1. Immunoassay Analyzer (ECL100) or (ECL25)
2. Immunoanalyzer Cuvettes (CL011) or (CL010)
3. EDI™ Wash Reagent (P-594)
4. EDI™Trigger Solution A and B (P-595A, P-595B)
5. EDI™q-FOBCollection Tube (30210)

SPECIMEN COLLECTION AND PREPARATION

Fresh fecal sample should be collected in a stool sample collection container by patients. It is advised to collect minimum of 1-2 mL liquid stool sample or 1-5 g solid stool sample. The sample should be transported to the lab in a frozen condition (-20ºC). Thesample is allowed to be stored at 2-8ºC if it is intended to be tested on a day of sample collection.

Fecal sample should be further collected and extracted in EDI™ qFOB Collection Tube (30210) in clinical laboratory. The tube is specifically designed for the easy collection/extraction of a substantial and consistent amount of a fecal sample into sample extraction buffer pre-filled tube. After collection, allow the tube to be sitting upright position for 3 - 10 minutes and then vortex the tube to dissolve all the feces. There should not have any feces stuck to the collection wand. Should the extracted sample be tested immediately, **please make sure that all the foam/bubble must be removed and the solid feces should be sedimented to the bottom of the tube**. A brief centrifugation procedure may be helpful to remove the bubble and to sediment fecal particles. The extracted fecal sample should be loaded on ECL-100 or ECL25 and should be tested within 6 hours.The extracted fecal sample may be stored below -20 °C for retention purpose. Avoid more than three freeze-thaw cycles for each specimen.

CALIBRATION

An active calibration curve is required for all tests. For the assay, calibration is required for the first time use of a reagent lot and every 14 days thereafter or when either kit control is out of range. Refer to appropriate system manuals for configuring calibrators.

QUALITY CONTROL

The characteristics of patient samples are simulated through controls and are critical to validate the performance of CLIA assays due to the random access format. Use of controls is left to the discretion of the user, based on good laboratory practices, requirements, and applicable laws. We suggest performing a control test once every day. Quality control results that do not fall within acceptable ranges may indicate invalid test results.

ASSAY PROCEDURE

1. Reagents from different kit lot numbers should not be combined or interchanged. Make sure that there are no air bubbles in any reagents, calibrator and control vials.
2. Reagent Preparation

2.1 Remove reagent cartridges from packaging and replace the solid caps with the provided soft caps for ECL100. For ECL25, carefully remove the aluminum foil seal on each container on the cartridges and insert soft caps.

2.2 For the ECL100, take out the Magnetic Particle bottle make sure to roll between hands and gently but thoroughly mix until the magnetic particle solution is homogenous. The solution should be uniform with no clumps of magnetic particles visible; this step is vital for assay performance. For ECL25, mix the magnetic beads by moving back and forth the bottom part of the cartridge at upright position. Make sure to look inside the cartridge until the solution is uniform with no clumps of magnetic particles visible and no air bubbles. Recap the bottle. Open the top soft cap of all reagent bottles, leaving only the hollow soft rubber. The reagents are now ready to be loaded into the ECL100 or ECL 25 for calibration.

3. Assay Program

The following table illustrates the protocol used by the ECL100 for instrument operation.

Component	Quality Control Hole (µL)	Sample Hole (µL)
q-FOB Controls (L0408-L0409)	40	-
Samples	-	50
Biotin q-FOB Antibody (L0402)	50	50
q-FOB Magnetic Particle Solution (L0401)	25	25
Incubation Period 1		
Wash the reaction cup 3 times with the wash solution.		
Acridinium Ester q-FOB Antibody (L0403)	75	75
Incubation Period 2		
Wash the reaction cup 3 times with the wash solution.		
Trigger Solution A (P-595)	200	200
Trigger Solution B (P-595)	200	200

The first result is less than 25 minutes after loading the tes

INTERPRETATION OF RESULTS

The chemiluminescence analyzer calculates the concentration values of the sample and the control by a standard curve (fitting method: four parameters or point-to-point) and the measured RLU. Values are compared with the range of the marked value. If it exceeds the indicated quality control range, it indicates that the test is unqualified and needs to be re-tested.

Due to methodological differences or antibody specificity, there may be deviations between the test results of reagents from different manufacturers. Therefore, direct comparisons should not be made to avoid false interpretation.

EXPECTED VALUES

Hemoglobin concentrations were measured in serum samples collected from 125 apparently healthy adults using the EDI™ Quantitative Fecal Occult Blood (q-FOB) CLIA Kit. The observed range of hemoglobin is summarized in the table below.

	Hemoglobin Concentration
Normal	0 - 50 µg/g
Light Positive	50 – 100 µg/g
Positive	100 – 200 µg/g
Strong Positive	>200 µg/g

It is highly recommended that each laboratory should establish their own normal range for hemoglobin based on local populations.

LIMITATIONS OF THE PROCEDURE

1. This product is for use on the ECL100 or ECL25 Immunoanalyzer only. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, operation, system performance, instructions, calibration, precautions, hazards, maintenance, and troubleshooting.
2. Reagents from different lots cannot be mixed.

3. Test results from this product should not be the sole basis for clinical diagnosis.
4. If the test sample result is higher than the upper limit of the calibration curve, it is recommended to re-measure after dilution according to a certain ratio. The measurement result is recalculated according to the dilution ratio to ensure the accuracy of the result.

PERFORMANCE CHARACTERISTICS

Hook Effect

The assay shows no hook effect up to 2,000 µg/g.

Limit of Blank

The limit of blank (LoB) was determined by 60 replicates of standard matrix to be 2.08 µg/g.

Limit of Detection

The limit of detection (LoD) was determined by 60 replicates of low-level samples to be 4.17µg/g.

Limit of Quantification

The limit of quantification (LoQ) was determined by 60 replicates of low-level samples to be 12.64µg/g.

Linearity

Linearity was determined by three replicates of each of the standards used to generate the multi-point calibration curve.

Standard	Average Concentration (µg/g)	Theoretical Concentration (µg/g)	R
1	48.2	64.9	0.99
2	117.5	129.9	
3	258.2	259.7	
4	373.2	389.6	
5	517.9	519.4	
6	645.4	649.3	

Repeatability

Repeatability was determined by measuring ten replicates of controls.

Standard	Average Concentration (µg/g)	CV (%)
1	41.233	3.2
2	13.996	3.6

Accuracy

Accuracy was determined by three replicates of two of the middle standards used to generate the multi-point calibration curve.

Standard	Average Concentration (µg/g)	Target Value ± 15% (µg/g)
3	20.7	17.5 – 23.7
5	195.7	157.3 – 212.8

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. F. Assche G. V. (2011). Fecal biomarkers for the diagnosis and management of inflammatory bowel disease. Gastroenterology & hepatology, 7(6), 396–398.
2. Bardhan PK, Beltinger J, Beltinger RW, Hossain A, Mahalanabis D, Gyr K (2000). "Screening of patients with acute infectious diarrhoea: evaluation of clinical features, faecal microscopy, and faecal occult blood testing". Scand. J. Gastroenterol. 35 (1): 54–60.doi:10.1080/003655200750024533. PMID 10672835
3. Beg M, Singh M, Saraswat MK, Rewari BB (2002). "Occult Gastrointestinal Bleeding: Detection, Interpretation, and Evaluation" (PDF). JIACM. 3 (2): 153–58. Archived (PDF) from the original on 2010-11-22.
4. E. Collins, Judith F.; Lieberman, David A.; Durbin, Theodore E.; Weiss, David G. (2005). "Accuracy of Screening for Fecal Occult Blood on a Single Stool Sample Obtained by Digital Rectal Examination: A Comparison with Recommended Sampling Practice". Annals of Internal Medicine. 142 (2): 81. doi:10.7326/0003-4819-142-2-200501180-00006. ISSN 0003-4819
5. Federici A, Giorgi Rossi P, Borgia P, Bartolozzi F, Farchi S, Gausticchi G. (2005). 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;12(2):83-8
6. Fraser CG, Matthew CM, Mowat NA, Wilson JA, Carey FA, Steele RJ. (2006). Immunochemical testing of individuals positive for guaiac faecal occult blood test in a screening programme for colorectal cancer: an observational study. Lancet Oncol;7(2):127-31.
7. Quintero E (2009). "[Chemical or immunological tests for the detection of fecal occult blood in colorectal cancer screening?]". Gastroenterol Hepatol (in Spanish). 32 (8): 565–76. doi:10.1016/j.gastrohep.2009.01.179. PMID 19577340.
8. Rabeneck, L., Rumble, R. B., Thompson, F., Mills, M., Oleschuk, C., Whibley, A. Lewis, N. (2012). Fecal immunochemical tests compared with guaiac fecal occult blood tests for population-based colorectal cancer screening. Canadian journal of gastroenterology = Journal canadien de gastroenterologie, 26(3), 131–147. doi:10.1155/2012/486328

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

Epitope Diagnostics, Inc.
7110 Carroll Road
San Diego, CA 92121, US

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

EC	REP

MDSS GmbH
Schiffgraben 41,
30175 Hannover, Germany

GLOSSARY OF SYMBOLS (EN 980/ISO 15223)