

How to Perform a Qualitative/Quantitative ELISA Assay to Detect *H. pylori* with the Thermo Scientific Multiskan FC Microplate Photometer

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Key words

- Qualitative ELISA
- Quantitative ELISA
- Thermo Scientific Multiskan FC
- Microplate Photometer
- Thermo Scientific SkanIt Software

Abstract

This application note describes how to perform both a qualitative and quantitative ELISA assay with a Thermo Scientific Multiskan FC microplate photometer. The kit used in this study was a Pyloriset® EIA-A III kit from Orion Diagnostica. The kit is an enzyme immunoassay for the detection and measurement of *Helicobacter pylori* (*H. pylori*)-specific IgA antibodies in human serum.

The assay can easily and reliably be performed with the Multiskan® FC Microplate Photometer using either the external or internal software.

Introduction

The Pyloriset EIA-A III is an enzyme immunoassay for the detection and measurement of *H. pylori*-specific IgA antibodies in human serum as an aid in diagnosing infection. The product is used to test patients with symptoms of gastrointestinal disorders and as a follow-up to medical treatment

In the assay, the serum samples are added to the microtiter wells coated with specific *H. pylori* antigens. If anti-*H. pylori* IgA is present in the specimen, it will bind to the antigen bound to the surface of the wells. The residual test specimen is washed away, and horseradish peroxidase-conjugated anti-human IgA reagent is added. Enzyme conjugate binds to an antigen-antibody complex, unbound conjugate is washed away, and a substrate is added. After stopping the substrate reaction, the color is measured using a photometer. The intensity of the color is proportional to the concentration of the *H. pylori*-specific antibody in the sample (Figure 1).

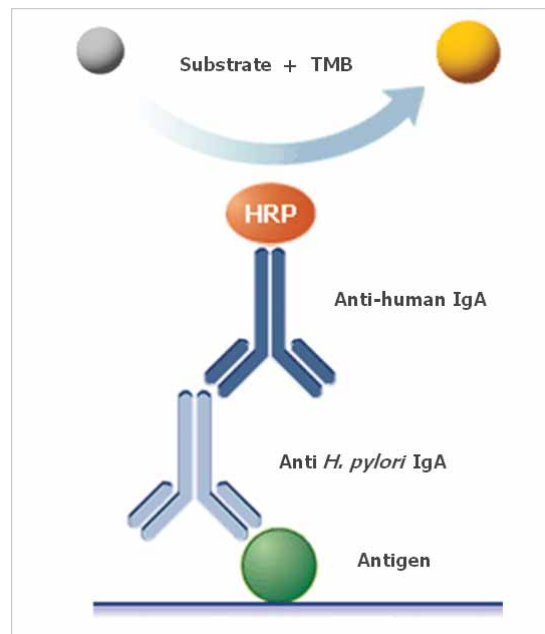


Figure 1. Basic principle of the Pyloriset EIA assay

Materials and Methods

- Pyloriset EIA-A III, *Helicobacter pylori* assay kit, Orion Diagnostica (Product no. 68107)
- Thermo Scientific Multiskan FC (Product no. 51119000 or 51119100)
- Photometric filter 450 nm (factory installed in Multiskan FC)
- Thermo Scientific Finnpiptette F1 (Product no. 4641070 Single Channel, 4661030 Multichannel)
- Thermo Scientific Wellwash 4 Mk 2 (Product no. 5160770)
- Thermo Scientific iEMS Incubator/Shaker (Product no. 5112200)

The Multiskan FC system includes two separate and alternative software programs. The Thermo Scientific SkanIt Software allows the user to operate the system via PC, while internal software enables keypad operation. In this study, the assay was performed using both the internal software and SkanIt® Software.

The assay was performed according to the kit instructions. The unknown samples were prepared by spiking the kit sample dilution buffer with Calibrator 3 in different concentrations. Calibrators were assayed in duplicates and unknown samples as singlicates.

The kit insert instructs the instrument to be blanked against air (without a plate). This is not necessary with the Multiskan FC, as the instrument automatically performs blanking during the measurement.

This kit provides an option to report either quantitative or qualitative results. Instructions are given here to set up a protocol for this assay on the Multiskan FC instrument with both types of software and both of the result reporting options.

The data processing parameters can be added either before or after the assay is run. In the example, all data processing is added beforehand. Protocols can also be downloaded from the Thermo Scientific Reading Room (www.thermo.com/readingroom). If downloaded, the layout must be modified according to the current layout and the filter information checked and/or changed according to the instrument configuration.

SkanIt Software for Multiskan FC

A new protocol can be created by selecting *New session* from the tool bar or from the Home view.

Layout

Create the layout according to the setup on the assay plate.

Protocol

Add a Photometric measurement step with the measurement mode *Normal* and the *450 nm* filter.

Results

SkanIt Software includes several ready-made calculations, including, quantitative and qualitative classification and quality control.

Add the Quality Control step under the Photometric (raw data) step.

The quality control rules of the kit state that the absorbance reading of the Calibrator Serum 1 should be below 0.200 and the absorbance reading of the Calibrator Serum 4 should be at least 0.800.

For SkanIt Software, the check is enabled by adding the rules:

1. Samples: Cal_0001 < 0.200
2. Samples: Cal_0004 > 0.800

This is how the formula appears with the default names. It is possible to change the name of the measurement step, calibrators, etc. The rule should be made according to the current assay naming convention. If these rules are not followed, the software will alert the user to check the assay results and repeat the test.

The quantitative calculation to create the standard curve and the concentrations of the unknown samples is enabled by adding a Curve Fit step to the raw data. The curve fit method used should be point-to-point in this assay.

Another option to report the results of this assay is the qualitative classification. In this case, the results of the unknowns are compared to the Calibrator Serum 2 (cut-off) and based on this classified as positive or negative (Figure 2).

For SkanIt Software this is done by adding a Qualitative Classification step to raw data and determining Calibrator 2 as limit 1. Both absorbance and concentration values can be used as the source data for the classification. Be sure to save the session.

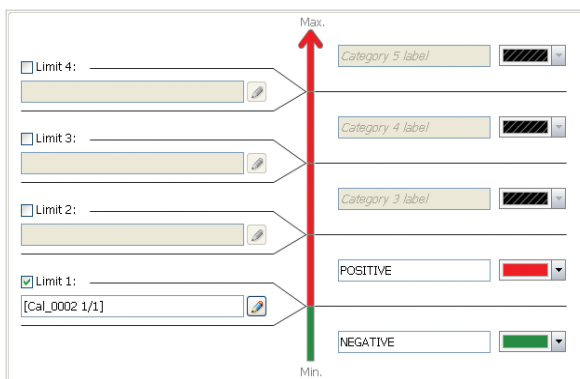


Figure 2. Qualitative classification in SkanIt Software

Multiskan FC Internal Software

A new protocol can be created by selecting *File/New* or changing the parameters of the current protocol and selecting *Save as*.

Main view

In the measurement parameters, select **450 nm** as filter 1 and the measurement mode **Normal**.

Processing

Create the layout according to the setup on the assay plate.

The quality control is enabled by selecting Yes in the Quality Control step.

For the internal software, the check is added by enabling quality control and adding the rules:

1. Sample: CAL1 raw, operator: < , constant: 0.200, the rule is shown as **Raw(CAL1) < 0.200**

2. Sample: CAL4 raw, operator: > , constant: 0.800, the rule is shown as **Raw(CAL4) > 0.800**

If these rules are not followed, the software alerts the user to check the assay results and repeat the test.

For the internal software, qualitative classification is added by enabling **Interpretation** and determining Calibrator 2 as limit 1 (Figure 3). Both absorbance and concentration values can be used as the source data for the classification.

Interpretation parameters	
Interpretation 3:	
Limit 2:	
Interpretation 2:	Positive
Limit 1:	Raw(CAL2)
Interpretation 1:	Negative
Source data:	Raw

Figure 3. Qualitative classification in the internal software

The quantitative calculation is enabled by enabling **Calculation** and selecting **Point-to-point** as the calculation type.

Results

Quantitative results

The results of the assay were calculated as instructed in the kit insert. A point-to-point curve was drawn with both pieces of software. The concentrations of the samples are automatically calculated from the curve and reported in list and table formats. An example of a calibration curve using SkanIt Software is shown in Figure 4.

Both pieces of software calculate and report the concentration of the samples with mean, standard deviation and CV% data.

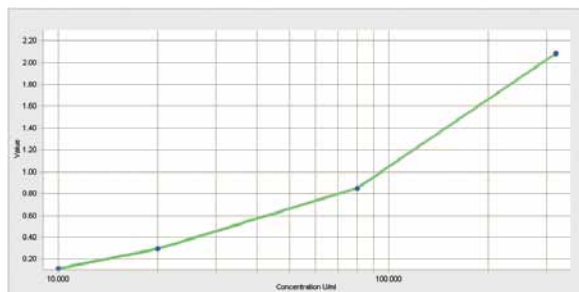


Figure 4. Example of a calibration curve in the Pyloriset assay using SkanIt Software for Multiskan FC

Qualitative results

The absorbance of each sample was compared to the absorbance value of Calibrator Serum 2.

If the U/ml or absorbance of the serum sample was

Well	Sample	Category	Value
E01	Un_0001 1/1	NEGATIVE	0.246
F01	Un_0002 1/1	POSITIVE	0.340
G01	Un_0003 1/1	NEGATIVE	0.154
H01	Un_0004 1/1	POSITIVE	0.444
A02	Un_0005 1/1	NEGATIVE	0.246

Figure 5. Example of qualitative results in the Pyloriset assay using SkanIt Software for Multiskan FC

equal to or higher than the Calibrator Serum 2, the sample was considered to be positive for H. pylori IgA antibodies. If the U/ml or absorbance of the serum sample was lower than the Calibrator Serum 2, the sample was considered to be negative for H. pylori IgA antibodies.

Both software packages calculate and report the interpretation of each sample.

SkanIt Software also offers color coding can also be used to make the interpretation more visual (Figure 5).

Conclusion

The Thermo Scientific Multiskan FC Microplate Photometer offers both PC and standalone users all of the data processing parameters needed for easy and reliable result reporting. This makes it an excellent tool for performing this type of assays.

Further Information

For further information about Multiskan FC, please refer to the following Web pages:

- www.thermo.com/readingroom
- www.thermo.com/mpi

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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