

CY-QUANT^ä ELISA sCD146

Enzyme immunoassay of soluble CD146
(sCD146, S-ENDO 1, MUC 18)

• *96-Test Kit Containing :*

- 3 x 2 Strips of Reagent 1 (Coated Strip)
- 3 vials of Reagent 2 (Calibrator)
- 6 Tablets of Reagent 3a (ortho-Phenylenediamine)
- 6 Tablets of Reagent 3b (Urea Peroxide)
- 1 vial of Reagent 4 (Dilution Buffer)
- 1 vial of Reagent 5 (Washing Solution)
- 3 vials of Reagent 6 (Anti- CD146- Peroxidase)
- 1 vial of Reagent 7 (Control plasma)
- 1 Plate Frame

Ref 7501



6 MATERIAL REQUIRED BUT NOT PROVIDED

- 3M sulphuric acid (the concentrated sulphuric acid is approximately 18M).
- Sterile deionized or distilled water.
- Timer.
- Multi-channel Pipette, pipette with disposable tips (10µl to 1000µl).
- Test Tubes.
- Plate washing equipment.
- Plate reader set at 492 nm.
- Vortex.

7 REAGENT PREPARATION AND STORAGE

Notes :

- Intact kits and contents remain stable until the expiration date printed on the box label, when stored at 2-8° C.
- All reagent must be kept at room temperature (18-25 °C) before use.
- Use good quality distilled water to dilute and reconstitute reagents.

• Reagent 1 :

Before opening, allow the reagent to stay at room temperature for 30 minutes. Then the strips are ready for use and the test must begin right after.

• Reagent 2 :

Ready for use

• Reagents 3a and 3b :

Prepare the OPD/ H₂O₂ substrate 5 minutes before use. For 2 strips dissolve **together** 2 tablets of Reagent 3a and 2 tablets of Reagent 3b in 8 ml of distilled water. This solution is stable for 1 hour at room temperature and in the dark.

Warning : handle OPD tablets with great care. Avoid contact with skin, wear gloves. Keep OPD tablets in their packaging and dissolve them only just before use in order to minimize spontaneous hydrolysis. Avoid all contacts with metallic surfaces as well as all contacts with oxidizing agents.

• Reagent 4*:

Before use, dilute the reagent 1:10 with distilled water.
Stability after dilution : 15 days at 2-8 °C when free of contamination.

• Reagent 5* :

Before use, dilute the reagent 1:20 with distilled water.
Stability after dilution : 15 days at 2-8 °C when free of contamination.

* **Note:** The presence of crystals will not affect the quality of Reagents 4 and 5. If necessary warm at 37 °C until all crystals have dissolved.

• Reagent 6 :

Reconstitute each vial with 8 ml of diluted Reagent 4. Allow the solution to stay at room temperature for 30 minutes.
Resuspend before use.

• Reagent 7 :

Reconstitute the vial with exactly 0.5 ml of distilled water. Allow the solution to stay at room temperature for 30 minutes.
Resuspend before use.
Stability after reconstitution : 4 hours at room temperature. It can be kept 15 days under frozen aliquots at -80°C.

Warning – Potential Biohazardous material

The reagents provided in this kit contain material of human and/or animal origin. Whenever human plasma is required for the preparation of these reagents, FDA-approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with regulatory safety precautions in the manipulations of these biological materials as if they were infectious.

For Research Use Only.

Not for Use in Diagnostic procedures.

1 METHOD

CY-QUANTTM ELISA sCD146 kit is an enzyme immunoassay (EIA) procedure for the determination of soluble CD146 (sCD146, S-ENDO 1, MUC 18) in plasma and serum.

2 EXPECTED VALUES

To determine.

3 SAMPLE

Serum, citrated or EDTA plasma.

4 TEST PRINCIPLE

A plastic support coated with specific mouse monoclonal anti-human CD146 F(ab)₂ fragments (Reagent 1) binds to the sCD146 to be measured. Next the mouse monoclonal antibody anti-CD146 coupled with peroxidase (Reagent 6) binds to the remaining free antigenic determinants of the CD146. The bound enzyme peroxidase is then revealed by its activity in a predetermined time on the OPD substrate (Reagent 3a) in the presence of urea peroxide (Reagent 3b). After stopping the reaction with a strong acid H₂SO₄ 3M, the intensity of the reaction is directly related to the concentration of sCD146 initially contained in the sample.

5 REAGENTS

- **Reagent 1 :** pouch containing 2 strips, each of 16 wells coated with specific mouse monoclonal anti-human CD146 F(ab)₂ fragments.
- **Reagent 2:** vial, 1 mL, recombinant human CD146 calibrator, in exactly known concentration (160 ng/ml).
- **Reagent 3a:** tablet containing 2 mg ortho-phenylenediamine (OPD, 2 HCl)
- **Reagent 3b:** tablet containing 5 mg urea peroxide.
- **Reagent 4:** vial, 20 mL, 10 fold-concentrated dilution buffer.
- **Reagent 5 :** vial, 50 mL, 20 fold-concentrated washing solution.
- **Reagent 6:** vial, specific mouse monoclonal anti- human CD146 antibody coupled with peroxidase, freeze dried.
- **Reagent 7 :** vial, freeze-dried human control plasma (control plasma) in exactly known concentration (see Assay Value Insert provided in the kit).

8 SPECIMEN COLLECTION AND TREATMENT

Sample collection:

- Anticoagulant :
 - Blood is collected in 0,109 M / 0,129M sodium citrate anticoagulant (using a ratio 9:1 vol.).
 - EDTA (K3).
- Centrifugation: 10 minutes at 2500g.
- Plasma storage: 8 hours at room temperature.

9 PROCEDURE

The kit provides sufficient reagents for a total of 96 determinations, which can be used in one, two or three time and enables the analysis of up to 41 samples in duplicate.

Calibration

Label 6 tubes, D1 to D6.

- In each of tubes D2 to D6, add the dedicated volume of Reagent 4 as indicated in the table below.
- In tube D1, pipette 1mL of Reagent 2.
- In tube D2, pipette 500 µL of tube D1 contents. Homogenize the tube using a vortex.
- Perform the subsequent serial dilutions as indicated in the table.

Change pipette tips after each addition to reduce contaminations.

Dilutions	R2 Volume	R4 Volume	Concentration *
D1	1000 µL	-	1 600 ng/mL
D2	500 µL de D1	500 µL	800 ng/mL
D3	500 µL de D2	500 µL	400 ng/mL
D4	500 µL de D3	500 µL	200 ng/mL
D5	500 µL de D4	500 µL	100 ng/mL
D6	-	500 µL	0 ng/mL

* **Note** : the concentrations indicated in this table already contain the 1:10 dilution.

Samples to be tested and control plasma

The samples to be tested, as well as the reconstituted control plasma must be diluted 1:10 with diluted Reagent 4. If high sCD146 levels are expected, then dilute the sample 1:20 with diluted Reagent 4.

Assay

Notes :

- During washing of strips, ensure that each well is completely filled with Reagent 5 and then completely emptied. The number of washing steps must be respected.
- Do not leave the wells dry at any time and if necessary fill the wells with Reagent 5.
- Do not expose the strips in strong light.
- Since the kinetics of the reaction is rapid the distribution of the calibration, the samples to be tested and the control plasma must be performed as quickly as possible. Equivalent incubation time for the different steps must be respected for each well.
- The washing steps can be performed either with plate washing equipment or with multi-channel pipette.

Just after opening the strips, distribute in duplicate :

- Calibration dilutions, diluted samples to be tested, reconstituted and diluted control plasma, within 4 hours after preparation.
- Reagent 4 (blank)

Pipette into each precoated well		
ANTIGEN IMMOBILIZATION	Diluted test sample	200 µL
	Incubate for 30 minutes at room temperature	
Wash all wells 5 times with diluted Reagent 5 then add immediately :		
IMMOBILIZATION OF IMMUNO- CONJUGATE	Reagent 6	200 µL
	Cover the wells and incubate 30 minutes at room temperature	
Wash all wells 5 times with diluted Reagent 5 then add :		
COLOR DEVELOPMENT	OPD/ H ₂ O ₂ Substrate (Reagents 3a + 3b)	200 µL
	Incubate at room temperature for 5 minutes* in the dark for each sample then add:	
	H ₂ SO ₄ 3M	50 µL
	Incubate for 20 minutes in the dark at room temperature	
LECTURE	Measure the absorbance at 492 nm (adjust reader to zero on blank Reagent)	

* the best absorbance at 492nm is located between 1 and 1.5 for the highest calibration point. To achieve this, adjust the time for hydrolysis.

10 RESULTS

Using a log-log graph paper, plot the sCD146 concentration values of the calibrator on the abscissa (x-axis) and their corresponding absorbance values on the ordinate (y-axis). Draw the calibration curve and interpolate the sCD146 concentration of the tested samples directly off the curve.

Ensure that the value of the reagent 7 (Control plasma) is within the range indicated in the Assay Value Insert provided in the kit. If the value is outside the stated range, all the results should be considered suspect. Check all components of the test system to ensure that all are functioning correctly, i.e. assay conditions, reagents, calibrations, integrity of the samples being tested, etc. If necessary repeat the test run.

Notes :

- The assay already includes the 1:10 dilution of the samples to be tested. Thus the sCD146 concentrations are read off directly from the curve.
- If greater dilutions are used, a corrective factor must be applied : the measured concentrations should be multiplied by D/10 (D = dilution factor).

11 PERFORMANCE CHARACTERISTIC

The use of F(ab)₂ fragments for the coating of the strips eliminates the interference by rheumatoid factor (RF).

12 REFERENCE

1- Kishimoto T. *et al.* Eds. 1996: Leucocyte Typing VI, Garland Publishing Inc, White Cell Differentiation Antigens pp755-759.

BIOCYTEX
140 CH. DE L'ARMÉE D'AFRIQUE
13010 MARSEILLE
FRANCE
TEL : +33 (0) 4 96 12 20 40
FAX : +33 (0) 4 91 47 24 71

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