CY-QUANT™ ELISA sCD146

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

- *96-Test Kit Containing :*

– 3 x 2 strips of Reagent 1 (Coated Strip)
– 3 vials of Reagent 2 (Calibrator)
– 1 vial of Reagent 3 (Washing solution)
– 1 vial of Reagent 4 (Dilution buffer)
– 1 vial of Reagent 5 (Anti CD146-peroxidase)
– 1 vial of Reagent 6 (TMB)
– 1 vial of Reagent 7 (Control plasma)
– 1 vial of Reagent 8 (Stop solution)
– 1 plate frame

Ref. 7501

BioCytex

For Research Use Only.
Not for Use in Diagnostic procedures.

1 METHOD

CY-QUANT™ ELISA sCD146 kit is an enzyme immunoassay (ELISA) procedure for the determination of soluble CD146 (sCD146, S-Endo, MUC 18) in plasma and serum.

2 EXPECTED VALUES

61 citrated plasma from healthy adults (men and women) were analyzed. These values are given as an indication only. It is recommended that each laboratory establishes its own normal values from a local population of normal donors.
Mean sCD146 = 273 ± 70 ng/mL

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 5) binds to a remaining free antigenic determinant of the CD146. The bound enzyme peroxidase is then revealed by its activity in a predetermined time on the TMB substrate (Reagent 6). After stopping the reaction (Reagent 8), the intensity of the signal 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 sCD146 calibrator, in exactly known concentration (160 ng/mL).
- **Reagent 3 :** vial, 50 mL, 20 fold-concentrated washing solution
- **Reagent 4 :** vial, 50 mL, dilution buffer.
- **Reagent 5 :** vial, 1.2 mL, 20 fold-concentrated specific mouse monoclonal anti human CD146 antibody coupled with peroxidase.
- **Reagent 6 :** vial, 25 mL TMB (tetra-methyl-benzidine).
- **Reagent 7 :** vial, freeze-dried human plasma (control plasma) in exactly known concentration (see Assay Value Insert provided in the kit).
- **Reagent 8 :** vial, 15 mL Stop solution.

6 MATERIAL REQUIRED BUT NOT PROVIDED

- Deionized or distilled water.
- Timer.
- Multi-channel pipette, pipette with disposable tips (10 µL to 1000 µL).
- Test tubes.
- Plate washing equipment.
- Plate reader set at 450 nm.
- Vortex.

7 WARNING

- Follow the conventional laboratory practices.
- Follow the appropriate regulation for waste disposal.
- Blood must be considered as potentially infectious.
- Reagent 4: Dilution buffer:
  H317: May cause an allergic skin reaction
  P280: Wear protective gloves / protective clothing / eye protection / face protection
  P302 + P352: IF ON SKIN: Wash with plenty of soap and water
- Reagent 7: Control plasma:
  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.
- Reagent 8: Stop solution:
  H314: Causes severe skin burns and eye damage
  P280: Wear protective gloves/protective clothing/eye protection/face protection
  P301 + P330 + P331: IF SWALLOWED: rinse mouth. Do NOT induce vomiting
  P303 + P361 + P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
  P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
  P310: Immediately call a POISON CENTER or doctor/physician

8 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 reagents 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. The test must begin right after opening of the pouch.

● Reagents 2, 4 and 8
Ready-to-use.

● Reagent 3 *
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 Reagent 3. If necessary warm at 37°C until all crystals have dissolved.

• **Reagent 5**
Before use, dilute the reagent 1:20 with Reagent 4.
Stability after dilution: 1 hour at room temperature when free of contamination.

• **Reagent 6**
Ready-to-use.
Note: **Avoid exposure to light, heat and contamination with metal ions or peroxidase.**

• **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.

9 SPECIMEN COLLECTION AND TREATMENT

Sample collection :
• Anticoagulant :
- 0.109M / 0.129M sodium citrate anticoagulant (using a ratio 9:1 vol.).
- EDTA (K3).

• Centrifugation : 10 minutes at 2500 g.
• Plasma storage : 8 hours at room temperature.

10 PROCEDURE

The kit provides sufficient reagents for a total of 96 determinations, which can be used in one, two or three times 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 (Dilution buffer) as indicated in the table below.
- In tube D1, pipette 1 mL of Reagent 2 (Calibrator).
- 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 avoid contaminations.

Dilutions	R2 Volume	R4 Volume	Concentration
D1	1000 µL	-	160 ng/mL
D2	500 µL de D1	500 µL	80 ng/mL
D3	500 µL de D2	500 µL	40 ng/mL
D4	500 µL de D3	500 µL	20 ng/mL
D5	500 µL de D4	500 µL	10 ng/mL
D6	-	500 µL	0 ng/mL

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 filled with 300 µL of diluted Reagent 3 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 diluted Reagent 3.
- 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 **300 µL diluted Reagent 3** then add immediately :		
IMMOBILIZATION OF IMMUNO-CONJUGATE	**Diluted Reagent 5**	**200 µL**
	Cover the wells and incubate **30 minutes** at room temperature	
Wash all wells **5 times** with **300 µL diluted Reagent 3** then add :		
COLOR DEVELOPMENT	**Reagent 6**	**200 µL**
	Incubate at room temperature for **5 minutes** for each sample then add:	
	Reagent 8	**100 µL**
	Incubate for **5 minutes** at room temperature	
LECTURE	Measure the absorbance at 450 nm (adjust reader to zero on blank reagent)	

11 RESULTS

Draw the calibration curve on a log-log scale by plotting the sCD146 concentration values of the calibrator on the abscissa (x-axis) and their corresponding absorbance values on the ordinate (y-axis). Interpolate the sCD146 concentration of the tested samples and control plasma (value to multiply by 10 to take the initial dilution into account).

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.

Note : if different sample dilutions are used, a corrective factor must be applied : the measured concentrations should be multiplied by 1/D (D = dilution factor).

12 PERFORMANCE CHARACTERISTIC

The use of $F(ab')_2$ fragments for the coating of the strips eliminates the interference by rheumatoid factor (RF).

13 REFERENCES

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BIOCYTEX
140 CH. DE L'ARMEE 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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