Version 03-06/25

User's Manual

Sirolimus ELISA Kit

REF **DEIA-WZ018**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The kit is intended for quantitative detection of sirolimus in whole blood sample. For research use only.

General Description

Sirolimus, also known as rapamycin, is a potent macrolide immunosuppressant with low toxicity and is commonly used in kidney transplants. The mechanism of action of sirolimus is different from that of other immunosuppressants and can be used to treat and reverse acute rejection, and prevent chronic rejection.

However, sirolimus has a long half-life, a narrow therapeutic window, and large individual variations in the pharmacokinetics and therefore, its improper use may cause adverse reactions, such as immune rejection, thrombocytopenia, leucopenia, or hyperlipidemia. Immunosuppressive drugs in the body are usually measured once or twice a day before the patient is given a new dosage and it is, therefore, important to monitor their blood levels for the duration of any treatment. In addition, a rapid and efficient monitoring method for sirolimus should allow its quantification in human whole blood, because it is mainly distributed in erythrocytes.

Reagents And Materials Provided

1. Microtiter plate with 96 (12×8) wells coated with coupling antigen
2. Sirolimus standard: 1 vials (0.2 ml, concentration see label)
3. Sample dilution: 1 vial (50 ml)
4. Antibody solution: 1 vial (7 ml)
5. Enzyme conjugate: 1 vial (7 ml)
6. Substrate: 2 vials (6 ml)
7. Stop Solution: 1 vial (7 ml)
8. Wash buffer (20×): 1 vial (50 ml)

Materials Required But Not Supplied

1. Microtiter plate spectrophotometer (450 nm/620 nm)
2. Polystyrene centrifuge tube
3. Micropipettes: 20 μL-200 μL, 100 μL-1000 μL, 250 μL-multipipette
4. Deionized water

Storage

Unopened kits can be stored stably at 2-8°C. Please refer to the kit label for expiration date.

Specimen Collection And Preparation

Notice and precautions for the users before operation:

a. Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.

b. Make sure that all experimental instruments are clean, otherwise it will affect the assay result.

Sample preparation:

1. Dilute the test sample 1:20 with the prepared sample diluent to get a final concentration.
2. Take 50 µL of the prepared solution for assay.

Reagent Preparation

1. To run the assay more than once, ensure that reagents are stored under the conditions stated on the label. Prepare only the appropriate amount necessary for each run.
2. Preparation of the wash buffer: Dilute the 20× concentrated wash solution with deionized water in the volume ratio of 1:19 (e.g. 10 ml of 20× concentrated wash solution + 190 ml of deionized water), which will be used for washing the plates. This solution can be stored at 4°C for 1 month.
3. Microtiter strips: After opening the sealed aluminum packaging, unused strips must be covered with adhesive foil and stored in the closed aluminum packaging together with desiccant at 2–8°C. We recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch. Opened microtiter plates are exposed to different conditions than sealed ones.
4. Preparation of the standard: Prepare a series of standards as shown in the figure below:

Suggested Preparation of Sirolimus Standards			
	ppb	Range: 0 to 40.5 ppb	
Stock	40500		
S1	40.5	Add 5µl Stock	+4995µl Dilution Buffer
S2	13.5	Add 100µl S1	+200µl Dilution Buffer
S3	4.5	Add 100µl S2	+200µl Dilution Buffer
S4	1.5	Add 100µl S3	+200µl Dilution Buffer
S5	0.5	Add 100µl S4	+200µl Dilution Buffer
S6	0.0		200µl Dilution Buffer

Note:

(1) The standard storage solution contains alcohol, so please accurately pipette the desired volume. Additionally, to avoid concentration inaccuracies due to evaporation, please store the solution tightly sealed.

(2) In addition to the standards provided in this kit, users may also use their own sirolimus standards. Please ensure that the sirolimus used is the same as that in the serum or plasma samples to be tested. The dilution method is also applicable as shown in the figure.

5. All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at 2–8°C.

Assay Procedure

Note:

1. Make sure all reagents and microwells are all at room temperature (20-25°C).
2. Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the reproducibility of the ELISA analysis.
3. Avoid the light and cover the microwells during incubation.

Procedure:

1. Take all reagents out at room temperature (20-25°C) for more than 30 min, homogenize before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. The wash solution should be brought to room temperature (20-25°C) before use.
4. Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
5. Add 50 µL of standard solution or prepared sample to corresponding wells.
6. Add 50 µL of enzyme conjugate solution, 50 µL of antibody solution to each well, mix gently by shaking the plate manually and incubate for 40 min at 25°C with cover.
7. Remove the cover gently and pour the liquid out of the wells and rinse the microwells with 300 µL diluted wash solution at interval of 10s for 5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).
8. Add 100 µL of substrate respectively to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25°C with cover.
9. Add 50 µL of the stop solution to each well. Mix gently by shaking the plate manually.
10. Measure the absorbance at 450nm against an air blank within 5 min after addition of stop solution. (It's suggested measure with the dual-wavelength of 450/630nm.) (We can also measure by sight without stop solution in short of the ELISA reader).

Calculation

Percentage absorbance: The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

Absorbance (%) = (B/B0)×100%

B - absorbance of standards or samples

B0 - absorbance of zero standard (0 ng/mL)

Precision

Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays), CV of the ELISA kit all less than 15%. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Sample	Intra Assay %CV	Inter Assay %CV
5	< 15%	< 15%

Sensitivity

The analytical sensitivity of the Sirolimus ELISA was found to be 0.31 ppb.

Specificity

The specificity was tested by measuring the cross-reactivity against tacrolimus, cyclosporine and everolimus.

The result indicates that the kit is specific for sirolimus.

Components	Cross-Reactivity
Sirolimus	100%
Tacrolimus	<0.02%
Cyclosporine	<0.02%
Everolimus	<0.4%

Precautions

1. The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25°C).
2. Do not allow microwells to be dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.
3. Mix the homogenate and elute the plate adequately.
4. Avoid the stop solution touching skin for the 2M H_2SO_4.
5. Don't use the kits out of date. Don't exchange the reagents of different batches, or else it will drop the sensitivity.
6. Storage constitution: Keep the ELISA kits at 2-8°C without frozen. Avoid direct sunlight during all incubations. Covering the microtiter plates is recommended.
7. The reagents go bad: Substrate solution should be abandoned if its color has changed. The reagents may be turn bad if the absorbance value (450/630 nm) of the zero standard is less than 0.5 (A450 nm < 0.5).
8. The coloration reaction needs 20-30 min after the addition of substrate; but you can prolong the incubation time.