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Quick Step Methotrexate mtx ELISA Kit

Size: 96 T, 48T

Catalogue Number:QS0046Ot

Assay Time: 60 minutes

Store all reagents at 2-8℃/-20°C

Validity Period: 2-8℃ for six months, -20°C for one year. Avoid repeated thaw cycles.

For samples: In serum, plasma, culture media or any biological fluid.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING！

Quick Step Methotrexate mtx ELISA Kit

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Purpose

Our Quick Step Methotrexate mtx ELISA Kit is to for the qualitative determination of Methotrexate mtx in Methotrexate serum, plasma, culture media or any biological fluid.

Principle

The ELISA is based on the the qualitative enzyme immunoassay technique.The Microplate provided in this kit has been pre-coated with an antibody specific to Methotrexate mtx, make it to solid-phase antibody.Samples are added to the Microplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for Methotrexate mtx is added to each Microplate well and incubated,so the antibody-antigen-Enzyme labeled antibody complex is formed.Following a wash to remove any unbound reagent,then the TMB substrate solution is added to each well. Only those wells that contain Methotrexate mtx and HRP conjugated Methotrexate mtx antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The qualitative determination of Methotrexate mtx is determined by comparing with the CUT OFF value.

Materials provided with the kit

	Materials provided with the kit	96 determinations	48 determinations
1	User manual	1	1
2	Closure plate membrane	2	2
3	Sealed bags	1	1
4	Microelisa stripplate	1	1
5	Negative control	0.5ml×1 bottle	0.5ml×1 bottle
6	Positive control	0.5ml×1 bottle	0.5ml×1 bottle
7	HRP-Conjugate reagent	6ml×1 bottle	3ml×1 bottle
8	Sample diluent	6ml×1 bottle	3ml×1 bottle

9	Chromogen Solution A	6ml×1 bottle	3ml×1 bottle
10	Chromogen Solution B	6ml×1 bottle	3ml×1 bottle
11	Stop Solution	6ml×1 bottle	3ml×1 bottle
12	Wash Solution	20ml (30X)×1bottle	20ml (20X)×1bottle

Sample collection and storages

1. Can't detect the samples which contain NaN3, because NaN3 inhibits HRP activity of the horseradish peroxidase.
2. Extract as soon as possible after Specimen collection, Extracted according to the relevant literature.

Cell culture supernates and plant exact fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw.

Notes:

Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20 ℃ . Repeated freeze-thaw cycles should be avoided.

Procedure

1. In the Microplate, number the corresponding micropores of the sample in sequence, leave two wells as negative control, two wells as positive control and one empty well as blank control. (Do not add samples and HRP-Conjugate reagent into blank control well, same for the rest steps)
2. **Add Samples:**Add 50μl of negative control and positive control into the designated negative control well and positive control well, respectively. Add 40μl Sample diluent and 10μl sample into the sample wells. Samples should be loaded onto the bottom

without touching the well wall. Mix well with gentle shaking.

3. **Add HRP:**Add 50µl HRP-Conjugate reagent to each well except the blank control well.
4. **Incubation:** Incubate 30 min at 37℃ after sealed with Closure plate membrane.
5. **Preparing solution:**Dilute the concentrated Washing Solution with distilled water (30 times for 96T and 20 times for 48T).
6. **Washing:**Carefully peel off Closure plate membrane, aspirate and refill with Wash Solution (350µl to 400µl, or fill it completely, overflow is acceptable). Remove the Wash Solution after resting for 30 seconds. Repeat the washing procedure for 5 times. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. **Chromogenic reaction:**Add 50µl Chromogen Solution A and 50µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 10min. **Protect from light.**
8. **Stop the reaction:**Add 50µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
9. **Assay:**Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Notes:

1. Store the kit at 4 °C upon receipt. The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from Methotrexate mtx antibody-Coated plate, reseal them in zip-lock foil and keep at 4 °C.
2. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
3. In order to avoid cross-contamination, Closure plate membranes are for one-time use only.
4. Please keep Substrate away from light.

5. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microelisa stripplate Reader.

6. All the samples, washing buffer and wastes should be treated as infectious agents.

7. Reagents from different lots should not be mixed.

Determine the result

Test effectiveness: the average value of positive control ≥1.00; The average value of negative control ≤0.10.

The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15

Negative judgement: if the OD value<CUT OFF, the sample is Methotrexate Methotrexate mtx negative.

Positive judgement: if the OD value ≥CUT OFF, the sample is Methotrexate Methotrexate mtx positive.

Storage and validity

1．Storage: 2-8℃/-20°C.

2．Duration: 2-8℃ for six months, -20°C for one year. Avoid repeated thaw cycles.