This package insert follows the Pharmaceuticals, Medical devices and Other Therapeutic Products Act of Japan.

In Vitro Diagnostics
Marketing Notification No. 13A2X00197225002

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This package insert must be read carefully prior to use.

Methotrexate assay kit for blood tests
(Classification No.: 30418001)

Nanopia eTDM Methotrexate

General Precautions

1. This product is for in vitro diagnostic use, and must not be used for any other purposes.
2. Clinicians should make a comprehensive clinical decision based on assay results in conjunction with clinical symptoms and other examination results.
3. Please read carefully the "PRECAUTIONS" section, particularly under "Effects on Laboratory Tests" of the package insert of each drug, for information on the influence to the assay result that medications administered to the patient have. Please also read carefully the "2) Cross-reactivity," under "2. Interfering Substances," in the "Procedural Precautions" section, as well as "2. Precautions for Assessment" in the "Assessment of Assay Results" section, of this package insert.
4. This product should be used only as directed in this package insert. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.
5. If the reagent accidentally comes in contact with eyes and/or mouth, rinse immediately with ample water as first aid, and consult the doctor if required.
6. Carefully read the operating instructions for each type of automated analyzers prior to using this product. Parameters for each type of analyzers are available, and can be requested from SEKISUI MEDICAL CO., LTD. if required.
7. Perform a quality control test prior to assay to ensure accuracy.

Description (Kit Components)

Component: Ingredients

MTX Antibody Solution 1:
Anti-methotrexate rabbit polyclonal antibody
Glucose-6-phosphate
Nicotinamide adenine dinucleotide (oxidized form)

MTX Enzyme Solution 2:
Glucose-6-phosphate dehydrogenase-labeled methotrexate

Intended Use

Measurement of methotrexate in serum or plasma

Methotrexate suppresses cell growth by inhibiting dihydrofolate reductase (DHFR), which reduces folic acid to produce the active form of folic acid required for nucleic acid synthesis, and inhibiting the thymidylic acid and purine synthesis enzyme systems. Methotrexate is used for methotrexate/leucovorin salvage therapy. Because certain cancer cells lack the ability to actively take up methotrexate, a high dose is administered so that excess methotrexate is passively incorporated. After a certain time, normal cells that can actively take up leucovorin are salvaged by administering leucovorin to reverse the toxicity of methotrexate. When methotrexate/leucovorin salvage therapy is performed, the risk of serious adverse events is high when the methotrexate concentration is not lower than 10, 1, and 0.1 μmol/L at 24, 48, and 72 hours after starting methotrexate treatment, respectively. Therefore, it is important to frequently measure the blood concentration of methotrexate for a certain period after administration.[1)]

Assay Principle

1. Assay Principle

This product is a homogenous enzyme immunoassay kit that employs a competitive reaction between methotrexate in a sample and glucose-6-phosphate dehydrogenase (G6PDH)-labeled methotrexate. When a certain amount of anti-methotrexate antibody is added to the sample, a reaction occurs and anti-methotrexate antibody is consumed depending on the amount of methotrexate in the sample. If G6PDH-labeled methotrexate is added, the residual anti-methotrexate antibody that has not bound to methotrexate in the sample binds to G6PDH-labeled methotrexate, while G6PDH-labeled methotrexate that does not bind to the anti-methotrexate antibody reduces NAD to NADH.

Since the amount of reduced NADH that is produced from NAD depends on the methotrexate concentration in the sample, the methotrexate concentration can be obtained by measuring the % change of absorbance. Because NAD only acts on the microorganism-derived enzyme used in this product, endogenous G6PDH in the sample has no influence on the assay results.

Sample (methotrexate) + Anti-methotrexate antibody ⟶ Antigen-antibody reaction

Unreacted anti-methotrexate antibody + G6PDH-labeled methotrexate
⟶ Antigen-antibody reaction

Glucose-6-phosphate + NAD
Unbound G6PDH-labeled methotrexate
⟶
6-phosphogluconate + NADH

2. Features

1) Liquid reagents, ready-to-use.
2) It can be measured using the automated analyzers commonly used.

Procedural Precautions **

1. Properties of Samples and Sampling Methods

1) Samples

Serum and plasma (heparin plasma and EDTA plasma) may be used.

2) Storage of samples

(1) After separation of serum (plasma), samples may be stored for up to 7 days in a refrigerator. If samples cannot be measured within 7 days of serum (plasma) separation, store them at −30°C and measure them within 35 days. Stored samples should be brought to room temperature (15–30°C) before use.

(2) Samples may be frozen and thawed up to twice.

3) Sampling should be performed after removing insoluble matter from the sample. Very cloudy specimens should be centrifuged before assay.

2. Interfering substances

1) Assay results are not affected by free bilirubin (up to 19.5 mg/dL), conjugated bilirubin (up to 20.9 mg/dL), hemoglobin (up to 491 mg/dL), formazin turbidity (up to 1410 FTU), or rheumatoid factors (up to 550 IU/mL).

2) Cross-reactivity

The following table summarizes the cross-reactivity with the methotrexate metabolites and other drugs.

(1) Methotrexate metabolites

Metabolite	Concentration tested (μmol/L)	Methotrexate concentration (μmol/L)	Cross-reactivity※ (%)
7-Hydroxy-methotrexate	5	0.05	< 0.02
	50	0.50	< 0.07
2,4-Diamino-N^{10}-methylpteroic acid (DAMPA)	0.05	0.00	< 100.0
	0.50	0.00	< 80.7

(2) Other drugs

If each drug is at the concentration shown in the table below when the methotrexate concentration is 0.00, 0.05, or 0.50 μmol/L, cross-reactivity is as follows.

Drug name	Concentration tested (μmol/L)	Cross-reactivity※ (%)
Adriamycin	1000	< 0.01
Cyclophosphamide	1500	0.00
Cytosine	1000	0.00
Dihydrofolic acid	1000	0.01
DL-6-Methyl-5,6,7,8-tetrahydropterin	1000	< 0.01
Folic acid	1000	< 0.01
Folinic acid (leucovorin)	1000	0.00
5-Fluorouracil	3000	0.00
6-Mercaptopurine	1000	0.00
5-Methyltetrahydrofolate	1000	0.00
Prednisolone	1000	0.00
Pyrimethamine	1000	0.00
Sulfamethoxazole	1600	0.00
Tetrahydrofolic acid	1000	< 0.01
Vinblastine	1000	0.01
Vincristine	1000	0.01
Triamterene	25	> 2.80
Trimethoprim	100	< 0.54

※Based on the Clinical and Laboratory Standards Institute (CLSI) EP7-A2, cross-reactivity was calculated by the following equation.

Cross-reactivity (%) = ({[Concentration of methotrexate in the sample after adding the metabolite or drug] – [concentration of methotrexate in the sample without the metabolite or concomitant drug]} / [Concentration of the metabolite or drug in the sample]) × 100

3. Others

1) Always use Methotrexate Calibrator for calibration.

2) Precautions for assay range

If the methotrexate concentration in the sample exceeds the measurement range, dilute the sample with a Methotrexate Sample Dilution Solution, and perform re-measurement.

Dosage/Administration (Assay Procedure) **

1. Preparation of reagents

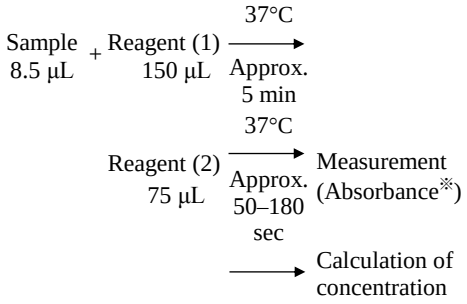
Reagent (1): MTX Antibody Solution 1 is ready to use.

Reagent (2): MTX Enzyme Solution 2 is ready to use.

Check that there are no bubbles with the reagents before using this product.

2. Assay Procedure

This product is compatible with various types of automated analyzer. An example of the assay procedure is indicated below.



※Absorbance : Absorbance at 340 nm

Calibration material: Methotrexate Calibrator (Manufacture's assigned value)

Assessment of Assay Results

1. Reference standard range

The 24-, 48-, and 72-hour values for blood methotrexate concentration limits for toxicity are specified as 10, 1, and 0.1 μmol/L, respectively.

2 . Precautions for Assessment

1) There may be reactions or interfering reactions with non-target substances. If assay results appear to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

2) This product shows cross-reactivity with DAMPA (a metabolite of methotrexate), accurate assay values cannot be obtained with samples collected from patients on gulcarpidase (carboxypeptidase G2) therapy.

Performance

1. Sensitivity
The difference between the change of absorbance per minute with the standard solution (0.00 μmol/L) and that with another standard solution (1.20 μmol/L) is ≥ 50.0 mAbs./min.

2. Accuracy: 80–120 % of the expected assay value

3. Within-run Reproducibility:
Coefficient of variation ≤ 15 %
(Test methods used for 1.–3. are in-house methods.)

4. Measurement Range[2]: (On Hitachi 7180 automated analyzer)
0.04–1.20μmol /L

5. Correlation[2]
1) Serum N=54 r=0.995 y=0.97x+0.011
Control method: Approved in vitro diagnostic (fluorescence polarization immuno assay)

6. Standard Material
Methotrexate (U.S. Pharmacopoeia)

Precautions for Use or Handling **

1. Precautions for Handling (to Ensure Safety)

1) All samples used in the test should be handled as a material possibly infected with HIV, HBV, HCV, or other viruses. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.
2) 2-Methylisoazolone is added as an antiseptic agent in the MTX Antibody Solution 1 and MTX Enzyme Solution 2. Therefore, if the reagent comes in accidentally contact with eyes, mouth or skin, rinse immediately with ample water as first aid, and consult the doctor if required.

2. Precautions for use

1) This product should be stored as directed, without freezing. Freezing can deteriorate the reagents, which can produce inaccurate results. Therefore, avoid using the reagents which have been previously frozen.
2) Do not use expired reagents. Use of such reagents cannot guarantee the reliability of measurement values.
3) Do not replenish the reagents.
4) After completion of measurement, this product should be tightly stoppered and stored in a refrigerator.
5) Do not mix materials from different kit lot numbers.
6) Do not perform the assay under direct sunlight

3. Precautions for Disposal

1) Before disposal, used samples and their containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1% for longer than 1 hour or autoclaved at 121ºC for 20 minutes.
2) To prevent infections from spilled samples or solutions containing samples, wipe the spilled area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration of greater than 0.1%.
3) The reagents and treated samples should be discarded as medical waste or industrial waste according to the waste disposal regulations.
4) The reagents should be disposed of in accordance with the Water Pollution Control act or related regulations.

4. Other precautions
Do not use the containers for other purposes.

Storage and Shelf Life **

1. Storage temperature: 2–8°C
2. Shelf life: 18 months from the date of manufacture
(The expiration date is printed on the outer package.)

Packaging

Name		Package
Nanopia eTDM Methotrexate	MTX Antibody Solution 1	1 × 16 mL
	MTX Enzyme Solution 2	1 × 8 mL

Constituent reagents are available in other configurations. For further details please contact SEKISUI MEDICAL CO., LTD.

References

1) Package insert of Methotrexate Injection 200mg･1000mg.
2) In house data, SEKISUI MEDICAL CO., LTD.

Contact

SEKISUI MEDICAL CO., LTD.
international@sekisui.com

Manufacturer **

SEKISUI MEDICAL CO., LTD.
1-3, Nihonbashi 2-chome, Chuo-ku, Tokyo, Japan