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

In Vitro Diagnostics
Certification No. 226ADAMX00197000

**Revised: October 2020 (3rd edition)
* Revised: January 2017 (2nd edition)

This package insert must be read carefully prior to use.

Tacrolimus assay kit
(Classification No.: 30421000)

Nanopia TDM Tacrolimus

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.
8. TACR Antibody Solution 1 contains human-derived ingredients that have been confirmed to be negative for HBs antigens, HIV antibodies, and HCV antibodies. However, handle this reagent with great care in the same manner as the samples, because it is potentially infectious.

Description (Kit Components)

Component: Ingredients
TACR Antibody Solution 1: Anti-tacrolimus rabbit monoclonal antibody
TACR Latex Reagent 2: Tacrolimus-coated latex
Pretreatment Solution 3

Intended Use

Measurement of tacrolimus in whole blood

Tacrolimus is a macrolide that has a strong immunosuppressive effect by selectively inhibiting the activation of T cells. Tacrolimus has been reported to cause adverse reactions such as infection, renal failure, and central nervous system disorders. Because there is considerable individual variation in the blood level of tacrolimus, it is necessary to measure blood concentrations and adjust the dosage depending on the condition of the patient. According to the Drug Interview Form, the therapeutic range is 5–20 ng/mL after organ transplantation (or 10–20 ng/mL after bone marrow transplantation when graft-versus-host disease is more likely to occur) and 10–15 ng/mL in patients with ulcerative colitis (reducing to 5–10 ng/mL after 2 weeks of treatment). It is mentioned that adverse reactions tend to occur in patients with persistently high blood trough concentrations (> 20 ng/mL).[1)]

Assay Principle

1. Assay Principle

Pretreat the whole blood sample with methanol and the Pretreatment Solution, and subsequently centrifuge. Use the supernatant thus obtained as the sample. When a certain amount of anti-tacrolimus antibody is added and reacted with a sample, consumption of the antibody depends on its content in the sample. When tacrolimus-coated latex is added, residual anti-tacrolimus antibody reacts with the latex and forms aggregates.
Since the extent of aggregation depends on the tacrolimus concentration in the sample, the tacrolimus concentration can be determined by measuring aggregation as the absorbance.

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

Unreacted anti-tacrolimus antibody + Tacrolimus-coated latex
⟶ Aggregation by antigen-antibody reaction

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
(1) Whole blood (EDTA-treated) is used as the sample.
(2) Use methanol (HPLC grade) and the Pretreatment Solution in the kit for pretreatment of the sample.
2) Storage of samples
(1) Samples may be stored for up to 7 days in a refrigerator. If samples cannot be measured within 7 days of collection, store them at −20°C or lower and measure them within 6 months. Stored samples should be brought to room temperature (15–30°C) before use.
(2) Samples may be frozen and thawed up to

twice.

3) Measure samples immediately after pretreatment.

2. Interfering substances

1) Assay results are not affected by free bilirubin (up to 19.1 mg/dL), conjugated bilirubin (up to 21.9 mg/dL), formazin turbidity (up to 1620 FTU), or rheumatoid factors (up to 500 IU/mL).

2) Cross-reactivity

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

(1) Tacrolimus metabolites

If each metabolite is at the concentration shown in the table below when the tacrolimus concentration is approximately 5–21 ng/mL, cross-reactivity is as follows.

Metabolite	Concentration tested (ng/mL)	Cross-reactivity ※ (%)
M-I (13-O-demethyl tacrolimus)	20	10.5
M-II (31-O-demethyl tacrolimus)	20	1.3
M-III (15-O-demethyl tacrolimus)	20	8.3
M-IV (12-hydroxy tacrolimus)	3.3	174.8
	20	10.5
M-VII (13,15-O-didemethyl tacrolimus)	20	11.5
M-VII (13,15-O-didemethyl tacrolimus) + M-VI (13,31-O-didemethyl tacrolimus)	20	4.0

(2) Other drugs

If each drug is at the concentration shown in the table below when the tacrolimus concentration is approximately 5 or 12 ng/mL, cross-reactivity is as follows. The recovery rate is 91–109%.

Drug name	Concentration tested (ng/mL)	Cross-reactivity ※ (%)
Acetaminophen	200,000	0.0
Acyclovir	1,000,000	0.0
Allopurinol	50,000	0.0
Amikacin sulfate	150,000	0.0
Amphotericin B	100,000	0.0
Ampicillin	100,000	0.0
Atenolol	40,000	0.0
Azathioprine	100,000	0.0
Azithromycin	5,000	0.0
Bromocriptine	8,000	0.0
Carbamazepine	120,000	0.0
Cefazolin	150,000	0.0
Ceftriaxone	500,000	0.0
Cephalosporin C	100,000	0.0
Chlorpromazine	50,000	0.0
Chloramphenicol	250,000	0.0
Chlordiazepoxide	20,000	0.0
Cimetidine	100,000	0.0
Ciprofloxacin	7,400	0.0
Clarithromycin	5,000	0.0
Clonidine	100	0.2
Colchicine	90	0.7
Cortisone	1,200	0.0
Cyclosporine A	10,000	0.0
Diazepam	20,000	0.0
Digitoxin	100,000	0.0
Digoxin	10,000	0.0
Diltiazem	60,000	0.0
Disopyramide	100,000	0.0
Erythromycin	200,000	0.0
Ethosuximide	300,000	0.0
Everolimus	100	–0.5
Famotidine	10,000	0.0
Fluconazole	100,000	0.0
Flucytosine	40,000	0.0
Furosemide	100,000	0.0
Ganciclovir	1,000,000	0.0
Gentamicin	120,000	0.0
Hydralazine	100,000	0.0
Hydrochlorothiazide	40,000	0.0
Ibuprofen	400,000	0.0
Itraconazole	100,000	0.0
Kanamycin A sulfate	100,000	0.0
Kanamycin B sulfate	100,000	0.0
Ketoconazole	100,000	0.0
Labetalol	17,100	0.0
Lidocaine	100,000	0.0
Lithium	35,000	0.0
Methylprednisolone	100,000	0.0
Metoclopramide	100,000	0.0
Minoxidil	60,000	0.0
Morphine sulfate	100,000	0.0
Mycophenolic acid	100,000	0.0
N-Acetylprocainamide	120,000	0.0
Nadolol	1,200	0.0
Naproxen	100,000	0.0
Nicardipine	500	0.1
Nicotine	20,000	0.0
Nifedipine	100,000	0.0
Penicillin G	100,000	0.0
Pentobarbital	100,000	0.0
Phenobarbital	150,000	0.0
Phenytoin	100,000	0.0
Prazosin	100,000	0.0
Prednisolone	100,000	0.0
Primidone	100,000	0.0
Probucol	600,000	0.0
Procainamide	100,000	0.0
Propranolol	40,000	0.0
Quinidine	100,000	0.0
Ranitidine	200,000	0.0
Rifampicin	100,000	0.0
Salicylic acid	500,000	0.0
Sirolimus	300	0.2
Spectinomycin	100,000	0.0
Streptomycin	100,000	0.0
Sulfamethoxazole	150,000	0.0
Theophylline	250,000	0.0
Ticlopidine	150,000	0.0
Tobramycin	100,000	0.0
Triamterene	100,000	0.0
Trimethoprim	40,000	0.0

Valproic acid	500,000	0.0
Vancomycin	100,000	0.0
Verapamil	100,000	0.0

※Based on the Clinical and Laboratory Standards Institute (CLSI) EP7-A2, cross-reactivity was calculated by the following equation.
Cross-reactivity (%) = ({[Concentration of tacrolimus in the sample after adding the metabolite or drug] – [Concentration of tacrolimus in the sample without the metabolite or concomitant drug]} / [Concentration of the metabolite or drug in the sample]) × 100

3. Others

1) Use Tacrolimus Calibrator for Nanopia as the calibration material after carrying out pretreatment in the same manner as for samples.
2) Precautions for assay range
If the tacrolimus concentration in the sample exceeds the measurement range, dilute the sample with Tacrolimus Calibrator A, and then perform pretreatment and re-measurement.

Dosage/Administration (Assay Procedure)

1. Preparation of reagents

Reagent (1): TACR Antibody Solution 1 is ready to use.
Reagent (2): TACR Latex Reagent 2 is ready to use.
Pretreatment Solution: Pretreatment Solution 3 is ready to use.
Before using this product, gently invert the TACR Latex Reagent 2 bottle to mix it thoroughly, and check that there are no bubbles.

2. Instruments and Reagents

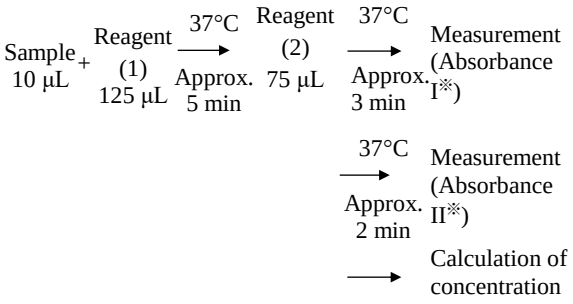
1) Micropipettes (10–100 µL and 100–500 µL)
2) Pipette tip
3) Methanol (HPLC grade)
4) Microcentrifuge tube
5) Vortex mixer
6) High-speed shaker (if used)
7) Microcentrifuge

3. Pretreatment of Samples

1) Gently mix the Pretreatment Solution and methanol (HPLC grade) at a volume ratio of 1:4 in a clean and dry air-tight bottle, and use this solution as the extraction solution (e.g., mix 10 mL of the Pretreatment Solution with 40 mL of methanol [HPLC grade]).
The extraction solution can be used for 2 weeks from the day of preparation when kept at room temperature.
2) Pipette 200 µL of the sample into a microcentrifuge tube.
3) Add 200 µL of the extraction solution and mix for 15–30 seconds in a Vortex mixer or for 5 minutes in a high-speed shaker.
4) After the microcentrifuge tube is let stand for 5 minutes at room temperature, perform centrifugation (13000 rpm for 5 minutes).
5) Dispense 200 µL of the supernatant thus obtained, and use it as the sample.

4. Assay Procedure

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



※Absorbance I and II: Absorbance at 700 nm
Calibration material: Tacrolimus Calibrator (Manufacture's assigned value)

Assessment of Assay Results **

1. Reference standard range

The therapeutic range of the whole blood concentration of tacrolimus is specified as 5–20 ng/mL after organ transplantation (or 10–20 ng/mL after bone marrow transplantation when graft-versus-host disease is more likely to occur) and 10–15 ng/mL in patients with ulcerative colitis (reducing to 5–10 ng/mL after 2 weeks of treatment).[1] However, the therapeutic range may overlap the toxic concentration range. Therefore, interpretation of assay results should also be based on the patient's clinical findings and other examination results.
It is recommended that the blood concentration of tacrolimus should be measured in a sufficient number of samples for statistical analysis and that its therapeutic range should be determined by each medical institution.

2. Precautions for Assessment

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.

Performance

1. Sensitivity

(1) Absorbance of standard solution (0.0 µg/mL) is ≥ 850 (Abs. × 10000).
(2) The ratio of absorbance between standard solution (0.0 ng/mL) and another standard solution (approx. 2.0 ng/mL) is ≤ 88%.

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)

1.5–30.0 ng/mL

5. Correlation[2]

1) Whole blood (EDTA)
N=168 r=0.932 y=1.03x+0.71
Control method: Approved in vitro diagnostic (chemiluminescence immunoassay)
2) Whole blood (EDTA)
N=93 r=0.926 y=1.06x+1.9
Control method: Approved in vitro diagnostic

(enzyme immunoassay)

6. Standard Material

Tacrolimus (in-house standard material)

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) Sodium azide is added as an antiseptic agent in the TACR Antibody Solution 1 and TACR Latex Reagent 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.
3) Methanol (HPLC grade) used for pretreatment is highly flammable liquid, and is known for strong eye irritancy, etc. When using Methanol, please handle it very carefully following its Material Safety Data Sheet.

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.
5) Sodium azide has been added as an antiseptic agent in the TACR Antibody Solution 1 and TACR Latex Reagent 2. It can react with lead or copper pipes to produce the highly explosive metal azide. Therefore, the reagent should be flushed with large amounts of water during disposal.

4. Other precautions

Do not use the containers for other purposes.

Storage and Shelf Life

1. Storage temperature: 2–8°C

2. Shelf life: 1 year from the date of manufacture (The expiration date is printed on the outer package.)

Packaging

Name		Package
Nanopia TDM Tacrolimus	TACR Antibody Solution 1	1 × 13 mL
	TACR Latex Reagent 2	1 × 8 mL
	Pretreatment Solution 3	2 × 15 mL

References

1) Drug Interview Form for Prograf Capsules 0.5mg, 1mg, 5mg, Prograf Granules 0.2mg, 1mg. Japanese.
2) SEKISUI MEDICAL CO., LTD. In house data. Japanese.

Contact

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