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

In Vitro Diagnostics
Marketing Approval No. 22400AMX01415000

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*Revised: January, 2017 (4th edition)

This package insert must be read carefully prior to use.

Everolimus assay kit
(Classification No.: 83022000)

Nanopia TDM Everolimus

Important Precautions

When measurement is changed from a conventional method (such as LC/MS/MS) to using this product, adequately assess differences from the values obtained by the conventional method and establish the appropriate target range that should be obtained by using this product at each medical institution.

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. EVER Antibody Solution 1 and Calibrators A to F are prepared from materials of human origin and human blood that have been shown to be negative for HBs antigen, HIV antibodies, and HCV antibodies. However, these reagents (as well as the specimens) should be considered potentially infectious and handled with great care.

Description (Kit Components)

Component: Ingredients
EVER Antibody Solution 1: Rabbit anti-everolimus polyclonal antibody
EVER Latex Reagent 2: Everolimus-coated latex
Pretreatment Solution 3
Calibrator A (CAL A)
Calibrator B (CAL B)
Calibrator C (CAL C)
Calibrator D (CAL D)
Calibrator E (CAL E)
Calibrator F (CAL F)

Intended Use

Measurement of the everolimus in whole blood

Assay Principle

1. Assay Principle

Pretreat the whole blood specimen with methanol and the pretreatment solution, and subsequently centrifuge. Use the supernatant thus obtained as the specimen. When a certain amount of anti-everolimus antibody is added to a specimen, the anti-everolimus antibody binds to everolimus until it has reacted with all of the available everolimus in the specimen. Next, everolimus-coated latex are added, which react with the residual anti-everolimus antibody, leading to agglutination. Since the extent of agglutination depends on the concentration of everolimus in the specimen, the everolimus concentration can be determined by measuring the absorbance as an indicator of agglutination.

Specimen (everolimus) + Anti-everolimus antibody
⟶ Antigen-antibody reaction

Residual anti-everolimus antibody + Everolimus-coated latex
⟶ Agglutination via an antigen-antibody reaction

2. Features

1) Liquid reagents, ready-to-use.
2) Applicable to various automated analyzers.

Procedural Precautions ** *

1. Properties of Samples and Sampling Methods

1) Samples
(1) Whole blood (EDTA) is used as the specimen.
(2) Pretreat specimens with methanol (HPLC grade) and the pretreatment solution in the kit.
(3) Measure specimens promptly after pretreatment, bearing the evaporation of methanol particularly in mind.

2) Storage of samples[1)]
(1) Specimens may be stored for up to 7 days in a refrigerator. If specimens cannot be measured within 7 days of collection, store them at -20°C and measure them within 28 days. Bring samples to room temperature (15–30°C) before use.
(2) Specimens may be frozen and thawed up to twice.
(3) Store specimens with protection from light, because light may affect stability.

2. Interfering substances

1) Assay results are not affected by free bilirubin

(up to 18.7 mg/dL), conjugated bilirubin (up to 19.7 mg/dL), formazin turbidity (up to 1440 FTU), rheumatoid factors (up to 360 IU/mL), γ-globulin (up to 12 g/dL), total protein (up to 12 g/dL), or hematocrit (up to 60%).

2) Cross-reactivity

Cross-reactivity with everolimus analogs and various other agents is detailed in the tables below.

(1)Everolimus metabolites

Substance	Concentration tested (ng/mL)	Cross-reactivity (%)
Seco acid of everolimus (RAD SA)	5	7
	20	2
Precursor of seco acid of everolimus (RAD PSA)	5	12
	20	16
40–phosphatidylcholine everolimus (RAD PC)	5	63
	20	59
45–/46–hydroxy everolimus (45/46 OH RAD)	5	ND
	20	2
24–hydroxy everolimus (24 OH RAD)	5	9
	20	5
25–hydroxy everolimus (25 OH RAD)	5	15
	20	22

(2) Sirolimus and its metabolites

Substance	Concentration tested (ng/mL)	Cross-reactivity (%)
Sirolimus	10	46
Trihydroxy sirolimus; 7, 41-O-didesmethyl sirolimus	90	4
41-O-desmethyl hydroxy sirolimus	90	3
41-O-desmethyl hydroxy sirolimus; 7-O-desmethyl sirolimus	90	2
11-hydroxy sirolimus	90	12
Isomer of 11-hydroxy sirolimus	90	6
Hydroxy sirolimus	90	2
N-oxide sirolimus	90	7
Isomer of hydroxyl sirolimus or n-oxide sirolimus	90	1
41-O-desmethyl sirolimus; 32-O-desmethyl sirolimus	30	45

(3) Drugs commonly prescribed with everolimus

Substance	Concentration tested (μg/mL)	Cross-reactivity (%)
Acetaminophen	200	ND
N-acetylprocainamide	120	ND
Acyclovir	1000	0.0
Albuterol	0.18	ND
Allopurinol	60	ND
Amikacin	150	ND
Amphotericin B	100	ND
Ascorbic acid	30	ND
Atenolol	40	ND
Azathioprine	10	ND
Caffeine	100	ND
Captopril	50	0.0
Carbamazepine	120	0.0
Cefaclor	230	ND
Chloramphenicol	250	ND
Cimetidine	100	ND
Ciprofloxacin	250	0.0
Cyclosporin A	1	ND
Digoxin	0.01	-2
Disopyramide	30	0.0
Erythromycin	200	0.0
Ethanol	3500	ND
Lithium	22.2	ND
Methicillin	240	ND
Methotrexate	910	ND
Morphine sulfate	6	ND
Mycophenolic acid	250	ND
Nadolol	333	ND
Naproxen	1000	0.0
Niacin	800	ND
Nifedipine	120	0.0
Omeprazole	14	ND
Penicillin G	100	0.0
Phenobarbital	150	ND
Phenytoin	100	0.0
Piperacillin	8	ND
Prazosin	25	ND
Prednisone	12	ND
Prednisolone	12	ND
Primidone	100	0.0
Procainamide	25	ND
Propranolol	0.5	ND
Quinidine	100	ND
Ranitidine	200	ND
Rifampin	50	0.0
Salicylic acid	500	ND
Spectinomycin	100	ND
Sulfamethoxazole	400	0.0
Tacrolimus	0.04	1
Theophylline	250	ND
Tobramycin	20	ND
Triamterene	600	0.0
Trimethoprim	20	ND
Valproic acid	1000	0.0
Vancomycin	630	ND
Verapamil	10	ND

ND: Not detectable

3. Others

1) Use Calibrators A to F as the calibration materials after carrying out pretreatment in the same manner as for specimens.
2) For quality control of this product, use Everolimus Control for Nanopia after

pretreatment in the same manner as for specimens. Everolimus Control for Nanopia® does not yield nominal values by other methods, such as LC/MS/MS. Do not use Everolimus Control for Nanopia for any purpose other than quality control of Nanopia TDM Everolimus.

3) Measurement range
If the everolimus concentration in a specimen exceeds the measurement range, dilute the specimen with Calibrator A (0.0 ng/mL), pretreat it, and perform re-measurement.

Dosage/Administration (Assay Procedure) ∗

1. Preparation of reagents

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

Reagent (2): EVER Latex Reagent 2 is ready to use.

Pretreatment solution: Pretreatment Solution 3 is supplied as a ready-to-use solution.

Calibrator A: Calibrator A should be used after being thawed at room temperature (15 to 30°C).

Calibrator B: Calibrator B should be used after being thawed at room temperature (15 to 30°C).

Calibrator C: Calibrator C should be used after being thawed at room temperature (15 to 30°C).

Calibrator D: Calibrator D should be used after being thawed at room temperature (15 to 30°C).

Calibrator E: Calibrator E should be used after being thawed at room temperature (15 to 30°C).

Calibrator F: Calibrator F should be used after being thawed at room temperature (15 to 30°C).

Before using this product, gently invert the EVER Latex Reagent 2 bottle to mix it thoroughly and check that there are no bubbles.

2. Instruments and Reagents

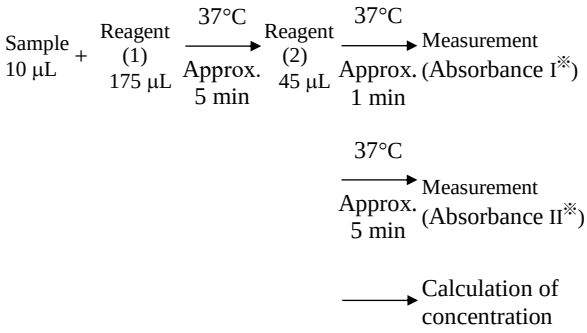
1) Micropipettes (10 to 100 μL and 100 to 500 μL)
2) Pipette tip
3) Methanol (HPLC grade)
4) Microcentrifuge tube with a cap
5) Vortex mixer
6) High-speed shaker (if used)
7) Microcentrifuge

3. Pretreatment of Specimens

1) Pipette 300 μL of a specimen into a microcentrifuge tube with a cap.
2) Add 350 μL of methanol (HPLC grade), cap the tube, and stir the contents with a Vortex mixer.
3) Add 50 μL of the pretreatment solution, cap the tube, and stir the contents for 35 seconds with a Vortex mixer or for 10 minutes with a high-speed shaker.
4) Place the capped microcentrifuge tube mentioned in section 3) above in the centrifuge (13400 × g for 10 minutes).
5) Pipette 350 μL of the supernatant thus obtained and use it as a specimen.

2. 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: Calibrators A to F (Manufacture's assigned value)

Assessment of Assay Results

1. Reference standard range

Based on the results of clinical trials, the recommended whole blood trough concentration of everolimus is 3 to 8 ng/mL (LC/MS/MS) for heart or renal transplant recipients, while it is 5 to 15 ng/mL (LC/MS/MS) for patients with subependymal giant cell astrocytoma associated with tuberous sclerosis complex (TSC-SEGA). However, therapeutic and toxic concentrations of this agent may overlap. Therefore, interpretation of assay results should also be based on the patient's clinical findings and other examination results.

It is recommended that the therapeutic concentration range of everolimus be determined at each institution by measuring the whole blood concentration in a statistically adequate number of specimens.

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 cross-reacts with metabolites of everolimus, and with sirolimus and its metabolites.

(3) Clinicians should make a comprehensive clinical decision based on assay results in conjunction with patients' clinical symptoms and other examination results.

(4) The recommended trough concentration range of everolimus (3 to 8 ng/mL) for heart or renal transplant recipients was based on the results of a non-Japanese phase III study (in patients with heart and renal transplantation) and a Japanese phase III study (in patients with renal transplantation) using LC/MS/MS for measurement.[1] The recommended trough concentration range of this drug (5 to 15 ng/mL) for patients with TSC-SEGA was based on the results of a non-Japanese phase II study using LC/MS/MS for measurement.[2),3)]

Clinical Significance **

Everolimus is a macrolide immunosuppressant that was developed from sirolimus (also called rapamycin and known to inhibit the immune system) in 1992. In Japan, everolimus was approved (brand name, Certican Tablets) in January 2007 for the prevention of graft rejection in heart transplant recipients and in December 2011 for the prevention of graft rejection in renal transplant recipients, and it is used to control immunosuppression after heart or renal transplantation. Common adverse reactions to everolimus include leukopenia, thrombocytopenia, and hyperlipidemia. The package insert states that the dose of this drug should be adjusted by monitoring the trough blood concentration because the blood everolimus concentration is influenced by food and the patient's condition. The package insert also states that the whole blood concentration of everolimus should be measured regularly when it is used in combination with ciclosporin. Furthermore, it states that the recommended trough blood concentration range of everolimus is 3 to 8 ng/mL, based on efficacy and safety analyses, and that neither efficacy nor safety has been evaluated at blood concentrations above12 ng/mL.

Everolimus was also approved for the treatment of TSC-SEGA in November 2012 (as Afinitor Tablets) and in December 2012 (as Afinitor Dispersible Tablets). The recommended trough blood concentration range is 5 to 15 ng/mL based on efficacy and safety analyses performed during a non-Japanese phase II study. Many patients with TSC-SEGA are relatively young children, who show marked individual differences of the blood everolimus concentration or also have epilepsy or other neurologic symptoms. Concomitant drugs may thus affect the blood concentration of everolimus in such patients. Therefore, the package insert states that the trough blood concentration of everolimus should be measured about 2 weeks after the start of administration or after changing the dose, and that it should be measured at appropriate times according to the patient's conditions that may affect the blood concentration of everolimus.

For other information including indications, effects, and dosage of everolimus drugs (e.g. Certican and Afinitor), please refer to the package insert of each drug.

Performance

1. Sensitivity

1) Absorbance of standard solution (0.0 ng/mL) is ≥ 900 (Abs. × 10000).
2) The difference of absorbance between standard solution (0.0 ng/mL) and another standard solution (1.5 ng/mL) is ≥ 90 (Abs. × 10000).

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[4]: (On Hitachi 7180 automated analyzer)

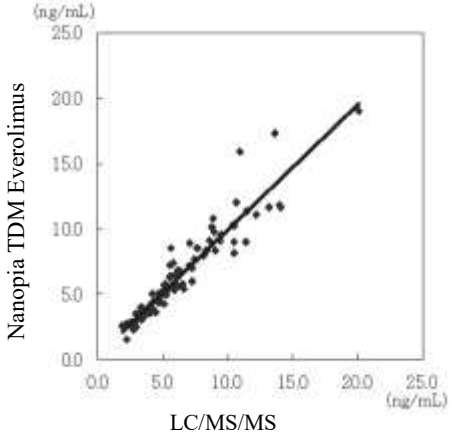
2.0–20.0 ng /mL

5. Correlation[4]

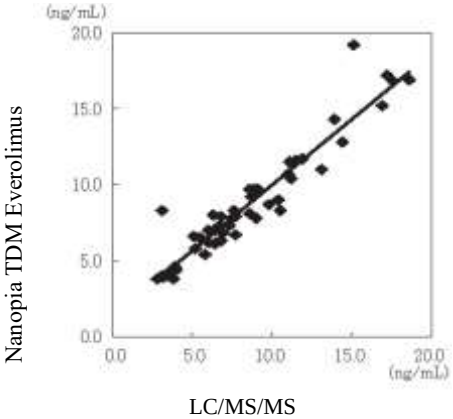
The correlation between values obtained with this product and those obtained by LC/MS/MS was assessed using specimens from transplant recipients at more than one institution after this product was marketed. The slope was 0.82 to 1.03, while it was 0.87 when the correlation was assessed using specimens from patients with TSC-SEGA. This product yields slightly lower values than those obtained by LC/MS/MS.

The correlation shown below is between values obtained with this product and values obtained by LC/MS/MS using specimens from transplant recipients and from patients with TSC-SEGA.

1) Whole blood (EDTA, specimens from heart transplant recipients)
N = 91, r = 0.946, *y* (obtained with Nanopia TDM Everolimus) = 0.96*x* (obtained by the reference method) + 0.31
Reference method: LC/MS/MS



2) Whole blood (EDTA, specimens from patients with TSC-SEGA)
N = 50, r = 0.943, *y* (Nanopia TDM Everolimus) = 0.87 *x* (LC/MS/MS) + 1.33



6. Standard Calibration Materials

Everolimus (in-house reference standard)
The calibration materials for this product are prepared by weighing everolimus (in-house reference standard) and adding it to whole human blood. Values of the calibration materials are obtained after correction using specimens from renal transplant recipients, the values of which are measured by LC/MS/MS at the reference institution.

Precautions for Use or Handling *

1. Precautions for Handling (to Ensure Safety)

1) All samples used in the test and Calibrators A to F 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 EVER Antibody Solution 1, EVER Latex Reagent 2, and Pretreatment Solution 3. 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) Proclin 300, which possesses skin-irritative potential, is added as an antiseptic agent in the Calibrators A to F. Therefore, if the reagent comes in contact with skin or clothes, rinse immediately with ample water, and consult the doctor if skin irritation develops.
4) Methanol (HPLC grade), which is used for pretreatment, is highly flammable and is known to severely irritate the eyes. Handle it with great care after reading the safety data sheet.

2. Precautions for use

1) EVER Antibody Solution 1, EVER Latex Reagent 2, and Pretreatment Solution 3 should be stored as directed in a tightly-stopped container. Avoid freezing. Do not use the product if it has been frozen because freezing can cause deterioration of the reagents, leading to inaccurate results.
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, reagents should be tightly stoppered and stored in a refrigerator.
5) Before use, Calibrators A to F should be thawed at room temperature (15 to 30°C), mixed by inversion, and pretreated.
6) After thawing, Calibrators A to F remain stable for 6 weeks when refrigerated (2 to 8°C) in tightly stoppered containers with protection from light.
7) Do not mix materials from different kit lot numbers.
8) Do not perform the assay under direct sunlight

3. Precautions for Disposal

1) Before disposal, used samples, Calibrators A to F, specimen containers, and calibrator containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1 % for longer than 1 hour or autoclaved at 121 ºC for 20 minutes.
2) To prevent infections from spilled samples, Calibrators A to F, or solutions containing Calibrators A to F or samples, wipe the spill area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration greater than 0.1%.
3) The reagents and treated samples, and residual Calibrators A to F should be discarded as medical waste or industrial waste according to the waste disposal regulations.
4) Pretreatment Solution 3 contains copper (II) sulfate. It should be disposed of in accordance with the Water Pollution Control Act or related regulations.
5) EVER Antibody Solution 1, EVER Latex Reagent 2, and Pretreatment Solution 3 contain sodium azide as a preservative. Sodium azide may react with lead or copper pipes and produce highly explosive metallic azide. When disposing of this product, flush it away with copious amounts of water.

4. Other precautions

Do not use the containers for other purposes.

Storage and Shelf Life *

The storage conditions and shelf-life of each kit component are as follows:

Components	Storage conditions	Shelf life
EVER Antibody Solution 1	2–8ºC	1 year from the date of manufacture
EVER Latex Reagent 2		
Pretreatment Solution 3		
Calibrator A (CAL A)	-25 to -15ºC (with protection from light)	1 year from the date of manufacture
Calibrator B (CAL B)		
Calibrator C (CAL C)		
Calibrator D (CAL D)		
Calibrator E (CAL E)		
Calibrator F (CAL F)		

(The expiration date is printed on the outer package.)

Packaging

Name		Packaging
Nanopia TDM Everolimus	EVER Antibody Solution 1	1 × 18 mL
	EVER Latex Reagent 2	1 × 5 mL
	Pretreatment Solution 3	1 × 5 mL
Everolimus Calibrator for Nanopia (sold separately)	Calibrator A (CAL A)	1 × 3.0 mL × 6 concentrations
	Calibrator B (CAL B)	
	Calibrator C (CAL C)	
	Calibrator D (CAL D)	
	Calibrator E (CAL E)	
	Calibrator F (CAL F)	

References

1) Novartis Pharma K.K. Japan, Certican Tablets 0.25 mg, 0.5 mg and 0.75 mg. [Instructions for Use]. Japanese.
2) Novartis Pharma K.K. Japan, Afinitor Tablets 2.5 mg and 5 mg. [Instructions for Use]. Japanese.
3) Novartis Pharma K.K. Japan, Afinitor Dispersible Tablets 2 mg and 3 mg. [Instructions for Use]. Japanese.
4) SEKISUI MEDICAL CO., LDT. In house date. Japanese.

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