Elecsys Everolimus

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REF		Σ	SYSTEM
07027257190	07027257500	100	**cobas e** 402 **cobas e** 801

English

System Information

Short name	ACN (application code number)
EVL	10126

Intended use

Immunoassay for the in vitro quantitative determination of everolimus in human whole blood. The assay is used as an aid in the management of kidney, liver and heart transplant patients receiving everolimus therapy.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Everolimus is a derivative of sirolimus often formulated for oral administration and is synthesized by the introduction of a 2-hydroxyethyl group at the carbon atom at position 40 of sirolimus.[1] The drug has many clinical applications, the most prominent being in organ transplantation, oncology and cardiology.[2] It has been shown that early replacement of calcineurin inhibitors such as cyclosporin with an everolimus based regimen may facilitate improved long-term outcomes in selected kidney transplant patients.[3]

Everolimus exerts its immunosuppressive and anti-proliferative effects through binding to FKBP-12 and subsequent inhibition of mTOR-mediated signaling, which is a mechanism identical to that of sirolimus. Everolimus displays the same activity in vivo as sirolimus.[2]

Upon entry into the cell, everolimus binds to the abundant immunophilin FKBP-12. The everolimus-FKBP-12 complex binds to mTOR which has the following two major functions: 1) activation of p70 S6 kinase, a key enzyme in signal transduction which leads to DNA synthesis, and 2) binding of the eukaryotic initiation factor 4E (eIF-4E) to phosphorylated heat- and acid-stable protein I (PHAS-I), a pathway that is more involved in protein synthesis. By binding to mTOR, everolimus blocks its function and thus inhibits activation of p70 S6 kinase, resulting in the arrest of the cell cycle at the G1 to S phase. Interleukin (IL)-2 receptor-dependent as well as CD28-dependent signaling pathways are inhibited by these effects on mTOR.[2,4,5]

The maximum concentration (c_{max}) of everolimus is reached within 1-2 hours following oral administration.[6] Similar to sirolimus, everolimus is a substrate of P-glycoprotein and CYP3A4. Therefore metabolism in the gastrointestinal tract and export back into the gut lumen can significantly affect overall bioavailability.[2] The parent drug is metabolized mainly in the liver and the gut by demethylation, hydroxylation, and ring degradation to form 6 main metabolites.[2] Approximately 75 % of circulating everolimus is bound to red blood cells and nearly 75 % of the remaining fraction is bound to plasma proteins.[2] The elimination half-life in renal transplant patients is 18-35 hours, which is approximately half that observed for sirolimus. The elimination half-life is slightly longer in liver transplant patients at 35-40 hours.[2]

When used in immunosuppressive therapy, the most common side effects associated with everolimus are peripheral edema, constipation, hypertension, nausea, anemia, urinary tract infection, and hyperlipidemia. Side effects also include increased risk of infection, development of lymphomas, graft thrombosis, delayed wound healing, nephrotoxicity, opportunistic infections and new onset diabetes after transplantation.[6]

Blood concentrations of everolimus in solid organ transplant patients correlate with therapeutic efficacy and frequency of adverse effects.[2] Due to the drug's narrow therapeutic range, the significant pharmacokinetic drug interactions and the high inter-patient variability, therapeutic drug monitoring (TDM) of everolimus within whole blood is therefore recommended for all solid organ transplant patients, and will likely result in an improved efficacy of the drug.[2,7,8,9]

Test principle

Manual precipitation:

Before testing with the Elecsys Everolimus assay, samples, calibrators and controls must be **pretreated** with Elecsys ISD Sample Pretreatment.

The reagent lyses the cells, extracts everolimus, and precipitates most of the blood proteins. The **pretreated** samples are centrifuged, and the resulting supernatant containing everolimus is then assayed using the Elecsys Everolimus assay.

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: By incubating 21 µL of the pretreated sample with an everolimus-specific biotinylated antibody, an immunocomplex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 2nd incubation: After addition of streptavidin-coated microparticles and an everolimus derivative labeled with a ruthenium complex[a)], the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as EVL.

M Streptavidin-coated microparticles, 1 bottle, 6.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-everolimus Ab~biotin, 1 bottle, 9.9 mL:
Biotinylated monoclonal anti-everolimus antibody (rabbit) 35 µg/L; phosphate buffer 100 mmol/L, pH 7.8; preservative.

R2 Everolimus derivate~$Ru(bpy)_3^{2+}$, 1 bottle, 6.8 mL:
Everolimus derivate labeled with ruthenium complex 18 µg/L; citrate buffer 10 mmol/L, pH 6.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

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Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

K_2-EDTA and K_3-EDTA whole blood.

Specimens collected in EDTA tubes may be stored for up to 5 days at 15-25 °C or 7 days at 2-8 °C prior to being tested. If testing will be delayed by more than 7 days, store frozen at -20 °C (± 5 °C) or lower for up to 6 months.

Freeze only once. Specimens must be mixed thoroughly after thawing to ensure consistency of the results.

Mix thawed specimens thoroughly by hand or on a roller mixer or rocker. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples and calibrators are at 20-25 °C prior to measurement.

Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.

Pretreated samples can be stored in closed tubes for up to 4 hours at 20-25 °C.

Due to evaporation effects, pretreated samples should be analyzed/measured within 30 minutes after opening the vials and loading the samples on the analyzer. Avoid delays between loading and measurement to ensure the 30 minute stability of pretreated samples.

A re-run requires repeating of the manual pretreatment procedure.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 05889073190, ISD Sample Pretreatment, 1 x 30 mL
- REF 06633196190, Everolimus CalSet, for 6 x 1.0 mL
- REF 07294131190, PreciControl Everolimus, for 3 x 3.0 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- REF 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer
- Precision pipettes (use only positive displacement pipettes for ISD Sample Pretreatment reagent handling)
- Microcentrifuge tubes (2.0 mL capacity)
- Microcentrifuge (at least 10000 g)
- Vortex mixer
- Roller mixer or rocker

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Manual specimen pretreatment

Follow the steps listed below to pretreat calibrators, controls and/or specimens. **The technical notes hereafter are an essential part of the instructions and must be read thoroughly before completing each step.** Follow steps 1 through 7 to pretreat calibrators, controls and/or specimens.

Steps	Technical notes
1. Equilibrate all reagents, calibrators, controls and specimens to 20-25 °C. Mix all calibrators, controls and specimens gently but thoroughly just before use.	Do not vortex. The liquids may be mixed by hand or on a roller mixer or rocker. The calibrators and controls are a whole-blood hemolysate and may be slightly different in appearance from whole-blood samples.
2. Label one microcentrifuge tube for each calibrator, control and/or specimen to be pretreated.	none
3. Using a precision pipette, transfer 300 µL of each calibrator, control and/or specimen to the appropriately labeled microcentrifuge tube.	Use a fresh pipette tip for each calibrator, control and/or specimen.
4. Using a precision pipette, add 300 µL of ISD Sample Pretreatment reagent to each microcentrifuge tube. Immediately cap each tube and immediately proceed to step 5.	Note: ISD Sample Pretreatment is highly volatile. Keep tightly closed when not in use to prevent evaporation.

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Steps	Technical notes
5. Vortex each microcentrifuge tube for at least 10 seconds. Failure to perform this step may result in a supernatant that appears red. See step 6, technical note.	Note: Failure to vortex each tube immediately after addition of the ISD Sample Pretreatment reagent will lead to erroneous assay results. Sample and reagent mixture should be completely homogeneous immediately after vortexing. Visual inspection is required.
6. Centrifuge the samples for at least 4 minutes in a microcentrifuge (≥ 10000 g).	The centrifuged samples should have well-defined pellets and clear supernatant. The supernatant should not appear cloudy or red. If the supernatant is red, discard and replace it with a newly extracted sample.
7. Transfer each supernatant directly into an appropriate vial and immediately cap each vial. The samples are ready to be assayed.	Pretreated samples can be stored in closed tubes for up to 4 hours at room temperature. **Please note:** **Due to evaporation effects, pretreated samples should be analyzed/measured within 30 minutes after opening the vials and loading the samples on the system. Avoid delays between loading and measurement to ensure the 30 minutes stability of pretreated samples.** **This is ensured by running the everolimus samples in batch mode:** **Based on average system sample processing time, no more than 35 everolimus samples may be loaded per calibrated measuring cell onto the analyzers at the same time.**

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This method has been standardized against gravimetrically produced master calibrators consisting of exactly defined pure substance everolimus concentrations in human whole blood matrix.

The predefined master curve is adapted to the analyzer using the relevant CalSet.

Everolimus CalSet must be pretreated freshly before calibration.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 28 days when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Everolimus.

PreciControl Everolimus must be pretreated freshly before measurement.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL, nmol/L or µg/L).

Conversion factors: ng/mL x 1.0 = µg/L

ng/mL x 1.044 = nmol/L

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Albumin	≤ 7.0 g/dL
Bilirubin	≤ 1129 µmol/L or ≤ 66.0 mg/dL
Biotin	≤ 287 nmol/L or ≤ 70.0 ng/mL
Cholesterol	≤ 500 mg/dL
HARA (human anti-rabbit antibodies)	≤ 10.0 µg/mL
Hematocrit	15-60 %
IgG	≤ 7.0 g/dL
IgM	≤ 1.0 g/dL
IgA	≤ 1.6 g/dL
Intralipid	≤ 2000 mg/dL
Rheumatoid factors	up to 1200 IU/mL
Uric acid	≤ 30.0 mg/dL

Criterion: For concentrations of 0.50-3.0 ng/mL the deviation is ≤ 0.60 ng/mL. For concentrations > 3.0 ng/mL the deviation is ≤ ± 20 %.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drugs were tested. Due to the cross-reactivity with sirolimus, a switch from one drug to the other might lead to overestimation of the blood levels of the currently administered immunosuppressant. Therefore, do not use samples from patients under sirolimus treatment or under transition from sirolimus to everolimus. The transition period may be approximated by the half-life of the eliminated drug where, for example, 12.5 % of a drug remains after 3 times the half-life.[6]

Special drugs

Drug	Concentration tested
Acyclovir	3.2 µg/mL

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Drug	Concentration tested
Amphotericin B	5.8 µg/mL
Ciprofloxacin	7.4 µg/mL
K_2-EDTA	6 mg/mL
K_3-EDTA	6 mg/mL
Erythromycin	20 mg/dL
Fluconazole	30 µg/mL
Flucytosine	40 µg/mL
Gancyclovir	1000 µg/mL
Gentamicin	12 mg/dL
Itraconazole	10 µg/mL
Kanamycin	100 µg/mL
Ketoconazole	50 µg/mL
Lidocaine	6 mg/dL
MPA (mycophenolic acid) glucuronide	1800 µg/mL
Mycophenolic acid	500 µg/mL
Nitrofurantoin	6 µg/mL
Phenobarbital	15 mg/dL
Spectinomycin	100 µg/mL
Sulfomethoxazole	200 µg/mL
Tacrolimus	60 ng/mL
Tobramycin	2 mg/dL
Trimethoprim	40 µg/mL
Vancomycin	6 mg/dL

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.5-30.0 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.5 ng/mL. Values above the measuring range are reported as > 30.0 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.4 ng/mL

Limit of Detection = 0.5 ng/mL

Limit of Quantitation = 1.0 ng/mL

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 25 %.

Dilution

Samples with everolimus concentrations above the measuring range can be manually diluted 1:2 with Diluent Universal prior to the manual pretreatment procedure. The concentration of the diluted sample must be > 12 ng/mL.

After manual dilution, multiply the result by the dilution factor.

Expected values

No firm therapeutic range exists for everolimus in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of everolimus, coadministration of other immunosuppressants, type of transplant, time post-transplant, and a number of other factors contribute to different requirements for optimal blood levels of everolimus. Individual everolimus values cannot be used as the sole indicator for making changes in the treatment regimen. Each patient should be thoroughly evaluated clinically before treatment adjustments are made, and each assay user must establish his or her ranges based on clinical experience.

These ranges will vary according to the commercial in vitro diagnostic test used. Ranges must be established for each commercial test used.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
HSP[b)] 1	1.33	0.068	5.1	0.081	6.1
HSP 2	2.53	0.102	4.0	0.129	5.1
HSP 3	7.43	0.220	3.0	0.323	4.3
HSP 4	14.8	0.375	2.5	0.589	4.0
HSP 5	28.5	0.847	3.0	1.30	4.6
PC[c)] Everolimus 1	3.64	0.128	3.5	0.167	4.6
PC Everolimus 2	9.22	0.284	3.1	0.373	4.0
PC Everolimus 3	19.0	0.566	3.0	0.735	3.9

b) HSP = Human Sample Pool

c) PC = PreciControl

Method comparison

a) A comparison of the Elecsys Everolimus assay, REF 06633188190 (y) with an automated immunoassay (x) using clinical samples gave the following correlations:

Number of samples measured: 151

Passing/Bablok[10]	Weighted Deming regression
y = 0.939x + 1.69	y = 1.05x + 1.03
τ = 0.753	r = 0.910

The sample concentrations were between 1.0 and 19.6 ng/mL.

b) A comparison of the Elecsys Everolimus assay, REF 06633188190 (y) with an LC-MS-MS method (x) using clinical samples gave the following correlations:

Number of samples measured: 184

Passing/Bablok[10]	Weighted Deming regression
y = 1.13x + 0.905	y = 1.20x + 0.580
τ = 0.840	r = 0.947

The sample concentrations were between 1.5 and 20.2 ng/mL.

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c) A comparison of the Elecsys Everolimus assay, REF 07027257190 (**cobas e** 801 analyzer; y) with the Elecsys Everolimus assay, REF 06633188190 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 163

Passing/Bablok[10]	Linear regression
y = 0.981x - 0.146	y = 0.997x - 0.286
τ = 0.947	r = 0.997

The sample concentrations were between 0.528 and 30.0 ng/mL.

d) A comparison of the Elecsys Everolimus assay, REF 07027257190 (**cobas e** 402 analyzer; y) with the Elecsys Everolimus assay, REF 07027257190 (**cobas e** 801 analyzer; x) gave the following correlations (ng/mL):

Number of samples measured: 154

Passing/Bablok[10]	Linear regression
y = 1.06x + 0.125	y = 0.986x + 0.547
τ = 0.958	r = 0.997

The sample concentrations were between 0.924 and 29.8 ng/mL.

Analytical specificity

Metabolite	Maximum concentration added ng/mL	Maximum cross-reactivity[d] %
24-Hydroxy everolimus	25	21.3
25-Hydroxy everolimus	25	15.4
45/46-Hydroxy everolimus	25	6.0
PKF 226-320 (RAD SA)	25	9.8
PKF 299-255 (RAD PSA)	25	10.1
RAD-PC	25	109.3

d) Representative data; results in individual laboratories may vary from these data.

References

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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