

REF



SYSTEM

07251254190

07251254500

300

cobas e 801

## English

### System information

Short name	ACN (application code number)
TCL	10022

### Intended use

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

The electrochemiluminescence immunoassay "ECLIA" is intended for use on the **cobas e 801** immunoassay analyzer.

### Summary

Tacrolimus (also referred to as FK506) is a macrolide antibiotic identified as a product of the actinobacterium *Streptomyces tsukubaensis* in Japan in 1984.<sup>1,2,3</sup> Studies demonstrated that tacrolimus is 10-100 times more active than cyclosporine at inhibiting several immune responses.<sup>4</sup>

The main mechanism through which tacrolimus exerts its immunosuppressive effect is believed to be via the inhibition of T cell activation and proliferation. Intracellular tacrolimus binds an immunophilin called FK506-binding protein (FKBP-12) and these complexes then inhibit the enzymatic activity of calcineurin.<sup>5</sup> The inhibition of calcineurin restricts the dephosphorylation and nuclear translocation of nuclear factor of activated T cells (NFAT), which regulates transcription of several cytokines, including IL-2, IL-4, TNF- $\alpha$ , and interferon- $\gamma$ , and therefore limits lymphocyte activation and proliferation.<sup>6,7,8,9,10</sup>

Tacrolimus is highly lipophilic and absorption is incomplete and variable. Following absorption, tacrolimus is highly bound to proteins and erythrocytes, with 99 % of the drug within the plasma being bound to albumin or  $\alpha$ -1-glycoprotein.<sup>11</sup>

The bioavailability and metabolism of tacrolimus are predominantly influenced by the activity of the cytochrome P450 isozymes CYP3A4 and CYP3A5, as well as the efflux pump p-glycoprotein, which show significant inter- and intra-individual variability in expression and function.<sup>12,13,14</sup>

Tacrolimus displays a high degree of inter- and intra-patient variability, as well as potentially severe side effects from doses that are either too low or too high. Inadequate tacrolimus concentrations might result in rejection of the transplanted organ. High levels may lead to severe adverse effects. Principle adverse effects associated with tacrolimus include nephrotoxicity, neurotoxicity, gastrointestinal disturbances, diabetogenesis, hypertension and malignant complications.<sup>15,16</sup>

The application of therapeutic drug monitoring (TDM) and concentration-controlled dosing in order to maintain each patient's drug exposure within a narrow therapeutic window is part of standard clinical practice for many years and is a major support to patient management.<sup>16,17</sup> Trough concentration (C<sub>0</sub>) monitoring is still widely used as a guide to individualizing tacrolimus dose requirements, even though some controversies remain about the relationship between C<sub>0</sub> and clinical outcome. The area under the concentration-time curve (AUC<sub>0-12</sub>) is generally considered the best marker of exposure but is expensive and impractical. To assess the efficacy of alternative strategies to C<sub>0</sub>, multicenter prospective trials would be needed.<sup>16</sup>

### Test principle

Manual precipitation:

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

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

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 21  $\mu$ L of pretreated sample is incubated with a tacrolimus-specific biotinylated antibody and a ruthenium complex<sup>a)</sup> labeled tacrolimus-derivate. Depending on the analyte concentration in the sample and the formation of the respective immune complex, the labeled antibody binding site is occupied in part with sample analyte and in part with ruthenylated hapten.
- 2nd incubation: After addition of streptavidin-coated microparticles, the 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)<sub>3</sub><sup>2+</sup>)

### Reagents - working solutions

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

- M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:  
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Anti-Tacrolimus-S-Ab~biotin, 1 bottle, 21 mL:  
Biotinylated monoclonal anti-tacrolimus-antibody (sheep) 15  $\mu$ g/L;  
phosphate buffer 100 mmol/L, pH 7.8; preservative.
- R2 Tacrolimus~Ru(bpy)<sub>3</sub><sup>2+</sup>, 1 bottle, 14.8 mL:  
Tacrolimus-derivate labeled with ruthenium complex 4  $\mu$ g/L; citrate  
buffer 10 mmol/L, pH 3.3; preservative.

### Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. 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:

2-methyl-2H-isothiazol-3-one hydrochloride

EUH 208 May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

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 <b>cobas e 801</b> analyzer	16 weeks

### Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

K<sub>2</sub>-EDTA and K<sub>3</sub>-EDTA whole blood.

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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.

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.

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.

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] 05889065190, Tacrolimus CalSet, for 6 x 1.0 mL
- [REF] 05889081190, PreciControl ISD, 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
- 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
- **cobas e 801** analyzer

Additional materials for the **cobas e 801** analyzer:

- [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 micro-centrifuge 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.
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 micro-centrifuge (≥ 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.





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Cross-reactivity was designated as „not detectable“ if the obtained value was less than the sensitivity of the assay.

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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.

## 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](http://dialog.roche.com) for definition of symbols used):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number

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