

# Cyclosporine Sample Pretreatment cobas®

COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent

REF 20766364 122

4 × 40 mL

## English

### System information

For use on COBAS INTEGRA analyzers the system ID is 07 6636 4.

### Intended use

The COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent is an in vitro diagnostic reagent intended for use with the COBAS INTEGRA Cyclosporine assay on the COBAS INTEGRA analyzers as an alternative to methanolic extraction of samples.

### Summary

The COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent is a liquid auxiliary reagent containing methanol used to extract cyclosporine from human samples in order to determine the cyclosporine concentration.

### Test principle

Before testing with the COBAS INTEGRA Cyclosporine assay, the samples, calibrators and controls are pretreated with the COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent. The reagent lyses the cells, extracts the cyclosporine and precipitates most of the blood proteins. The pretreated samples are centrifuged, and an aliquot of the resulting supernatant containing cyclosporine is then assayed using the COBAS INTEGRA Cyclosporine Assay.

### Reagents - working solutions

Contains: Cupric sulfate, methanol (27 % w/w), ethylene glycol (38 % w/w), TRIS buffer, sodium azide (< 0.05 % w/w), streptomycin (< 0.005 % w/w) and surfactant.

### 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.

For USA: For prescription use only.

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



Danger

H226 Flammable liquid and vapour.

H301 Toxic if swallowed.

H312 + H332 Harmful in contact with skin or if inhaled.

H370 Causes damage to organs.

### Prevention:

P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking.

P233 Keep container tightly closed.

P240 Ground/bond container and receiving equipment.

P241 Use explosion-proof electrical/ventilating/lighting/equipment.

P242 Use only non-sparking tools.

P243 Take precautionary measures against static discharge.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P264 Wash skin thoroughly after handling.

P270 Do not eat, drink or smoke when using this product.

P271 Use only outdoors or in a well-ventilated area.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

### Response:

P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.

P303 + P361 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

P304 + P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

P307 + P311 IF exposed: Call a POISON CENTER or doctor/physician.

P330 Rinse mouth.

P363 Wash contaminated clothing before reuse.

P370 + P378 In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction.

### Storage:

P403 + P235 Store in a well-ventilated place. Keep cool.

P405 Store locked up.

### Disposal:

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

Product safety labeling primarily follows EU GHS guidance.

The reagent contains cupric sulfate and sodium azide. Sodium azide may react with lead and copper plumbing to form potentially explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

### Reagent handling

The COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent is provided ready to use. Keep the bottle tightly capped when not in use.

### Storage and stability

Store the reagent at room temperature (18-25 °C). Keep away from sources of ignition. The reagent is stable up to the expiration date printed on the label, if stored as directed. Discard after expiration date. Improper reagent storage can affect assay performance.

### Specimen collection and preparation

Patient samples being tested should not exceed 55 % hematocrit.

The required sample volume is 100 µL of whole blood.

Pharmacokinetic factors influence the correct time of sample collection after the last drug dose. A trough sample is recommended for measurement of cyclosporine.

Blood should be drawn using tubes containing the anticoagulant EDTA. EDTA is recommended as the anticoagulant of choice for assaying cyclosporine in whole-blood samples. Heparinized samples are not recommended because they may form clots during storage.

Use fresh samples. If samples are to be tested within 8 hours of collection, they may be stored at room temperature (18-25 °C). They may be stored refrigerated at 2-8 °C for up to one week. If longer storage is necessary, samples should be frozen at -20 °C. Cyclosporine has been shown to be stable in whole-blood samples for at least 3 months when stored at -20 °C<sup>1, 2</sup>. Thaw and thoroughly mix frozen samples before testing. Repeated freeze-thaw cycles should be avoided. Aspiration of insoluble materials that

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## COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent

may form when some samples are frozen should be avoided when pipetting.

### Materials provided

See "Reagents – working solutions" section for reagents.

### Materials required (but not provided)

Multilevel whole-blood controls

COBAS INTEGRA analyzer

COBAS INTEGRA Cyclosporine **cobas c** pack

Cyclosporine Calibrators

Precision pipettes

Microcentrifuge tubes (1.5 to 2.0 mL capacity)

Microcentrifuge

Microcentrifuge tube rack

Vortex mixer

Sample inverter or rocker (optional)

### Assay

#### Pretreatment procedure

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

Follow Steps 1 through 8 to pretreat calibrators, controls and/or samples.

Steps	Technical notes
1. Equilibrate all reagents, calibrators, controls and samples to room temperature (18-25 °C). Mix all calibrators, controls and samples gently but thoroughly just before use.	Do not vortex. The liquids may be mixed by hand or on an inverter or rocker.  The calibrators 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 sample to be pretreated.	(none)
3. Using a precision pipette, transfer 100 µL of each calibrator, control and/or sample to the appropriately labeled microcentrifuge tube.	A single capillary tube may be used to dispense all of the calibrators, controls and samples, provided that the outside of the capillary barrel and the plunger tip are thoroughly wiped between samples with a moist laboratory tissue.
4. Using a precision pipette, add 300 µL of COBAS INTEGRA Cyclosporine Sample Pretreatment Reagent to each microcentrifuge tube. Immediately cap each tube.	(none)
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 7, Technical note.	Sample plus reagent mixture should be completely homogeneous immediately after vortexing.
6. Incubate the contents of the microcentrifuge tubes at room temperature for at least 2 minutes after vortexing of the last sample is completed.	Microcentrifuge tubes may be incubated for up to 10 minutes after vortexing and before centrifuging.

Steps	Technical notes
7. Centrifuge the samples for at least 2 minutes in a microcentrifuge (g force x minutes ≥ 25,000 g-min).	The centrifuged samples should have well-defined pellets and clear supernatant. The supernatant may appear slightly blue or green, but it should not be cloudy. If the supernatant is cloudy or becomes cloudy upon standing, it should be recentrifuged. The supernatant should not appear red. If the supernatant is red, discard and replace it with a newly extracted sample.
8. Decant each supernatant directly into a COBAS INTEGRA sample cup and immediately cap each cup. The samples are ready to be assayed.	The supernatants should be used immediately.

### Diluting high-concentration samples

If a pretreated patient sample assays higher than 500 ng/mL (416.3 nmol/L) cyclosporine, use the following directions to dilute the original whole-blood sample using the COBAS INTEGRA Cyclosporine Calibrator 0 ng/mL.

- Using an inverter, mix the original whole-blood sample and the COBAS INTEGRA Cyclosporine Calibrator 0 ng/mL gently but thoroughly just before use.
- Combine one part whole-blood sample with two parts COBAS INTEGRA Cyclosporine Calibrator 0 ng/mL. Be sure to prepare at least 100 µL diluted sample.
- Mix the diluted whole-blood sample gently but thoroughly by repeated inversion.
- Pretreat the diluted whole-blood sample using Steps 1 through 8 of the Pretreatment procedure.
- Assay the sample and multiply the result by 3 to obtain an estimate of the cyclosporine concentration.




### References

- Schran HF, Rosano TG, Hassell AE, et al. Determination of cyclosporine concentrations with monoclonal antibodies. Clin Chem 1987;33:2225-2229.
- Wong PY, Mee AV, Glenn J, et al. Quality assessment of cyclosporine monitoring Canadian validations. Transplant Proc 1990;22:1216-1217.

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.

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

### FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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