ARK™ Methotrexate II Assay
Roche cobas® c 503 Analytical Unit

For In Vitro Diagnostics Use

INTENDED USE

The ARK Methotrexate II Assay is a homogeneous enzyme immunoassay intended for the quantitative determination of methotrexate in human serum or plasma on automated clinical chemistry analyzers. The measurements obtained are used in monitoring levels of methotrexate to help ensure appropriate therapy.

Specimens obtained from patients who have received glucarpidase (carboxypeptidase G2) as a high dose methotrexate rescue therapy should not be tested with the ARK Methotrexate II Assay.

ADDITIONAL INFORMATION

The following are User-Defined Parameters for use when performing the ARK Methotrexate II Assay for measurement of methotrexate in human serum or plasma on the Roche cobas® pro chemistry systems. The Roche cobas c 503 analytical unit is available on the pro system. Instrument operating instructions are contained in the Roche cobas pro system operator's manuals.

Please review **IMPORTANT INFORMATION** below. Refer to applicable package inserts for information regarding intended use, reagent storage, specimen handling, calibration, quality control and other required information. ARK package inserts for reagent, calibrator, control and dilution buffer are available online at www.ark-tdm.com.

ORDERING INFORMATION

Product Name	Quantity/Kit	ARK Product Number
ARK Methotrexate II Assay	R1 16 mL, R2 8 mL	5071-0001-00
ARK Methotrexate II Assay	R1 28 mL, R2 14 mL	5071-0001-01
ARK Methotrexate II Calibrators	6 x 4 mL	5071-0002-00
ARK Methotrexate II Control	3 x 4 mL; LOW, MID, HIGH, 3 x 2 mL; 5, 50, 500 µmol/L	5071-0003-00
ARK Methotrexate II Control	3 x 4 mL; LOW, MID, HIGH	5071-0003-01
ARK Methotrexate II Control	3 x 2 mL; 5, 50, 500 µmol/L	5071-0003-02
ARK Methotrexate II Dilution Buffer	25 mL	5071-0004-00

Required but not provided (Contact Roche Diagnostics)

Roche cobas c pack green

Roche cobas c pack MULTI open/close tool

For Customer Support, contact ARK Diagnostics, Inc.

ARK Diagnostics, Inc., Fremont, CA 94538
customersupport@ark-tdm.com
Toll-free Tel: 1-877-869-2320
www.ark-tdm.com

PREPARATION OF ASSAY COMPONENTS

The following assay components are ready-to-use liquids as supplied from ARK Diagnostics. When not in use, store upright at 2-8°C. Components are stable until the expiration date printed on the label if stored as directed.

Reagent preparation and cobas c pack green assembly.
Reagent handling – ready to use
Reagent R1: Antibody/Substrate and **Reagent R2:** Enzyme.
Precaution: Avoid cross-contamination of R1 and R2.

*Filling the **cobas** c pack green*

1. Turn the cobas c pack green toward you.
2. Position B of the cobas c pack green is on the left side and position C on the right side.
3. Unscrew the screw cap of the bottle in position B on the left side of the cobas c pack green using the Open/Close tool.
4. Use a funnel to pour the whole contents of the R1 bottle into the opened bottle of the cobas c pack green (position B). Discard the funnel.
5. Close the bottle tightly using the Open/Close tool.
6. Unscrew the screw cap of the bottle in position C on the right side of the cobas c pack green using the Open/Close tool.
7. Use a funnel to pour the whole contents of the R2 bottle into the opened bottle of the cobas c pack green (position C). Discard the funnel.
8. Close the bottle tightly using the Open/Close tool.

The cobas c pack green is now ready for use.

NOTE: Solutions must be at the reagent compartment storage temperature of the analyzer before performing assays. Always use a new cobas c pack green when preparing fresh reagent. Never reuse accessories designed for single use, as this may result in reagent contamination and could affect test results. If the cobas c pack green bottles are not filled correctly, this may result in faulty reagent pipetting and could cause erroneous results.

Calibrators, Controls and Dilution Buffer: Supplied separately. Perform assay-specific calibration and quality control as recommended.

SPECIMEN COLLECTION AND PREPARATION

Refer to the ARK Methotrexate II Assay package insert for information on specimen collection and preparation.

Precaution: ARK Diagnostics has not validated saline or water as sample diluents. The use of alternate diluents and dilution protocols for specimens containing high concentrations of methotrexate should be validated by the end-user beforehand.

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DILUTION PROTOCOL

The measurement range of the ARK Methotrexate II Assay is 0.030 – 1.300 µmol/L. Specimens and controls containing methotrexate in higher concentrations (>1.300 µmol/L) are assayed by dilution of the specimen and controls into the measurement range. Specimens or controls may be diluted by using the ARK Methotrexate II Dilution Buffer. Prepare the appropriate ten-fold serial dilution as shown below. Multiply the assayed result by the dilution factor.

Volume	Sample	Dilution Buffer Volume	Dilution	Dilution Factor
50 µL	Undiluted sample	450 µL	1:10	10
50 µL	1:10 sample	450 µL	1:100	100
50 µL	1:100 sample	450 µL	1:1000	1000

ARK Diagnostics has not validated Roche saline as a sample diluent for use in the auto dilution procedure but the suggested auto dilution instrument programming is provided below. Auto dilution programming must be validated prior to reporting patient results. The use of alternate diluents and dilution protocols for specimens containing high concentrations of methotrexate should be also be validated by the end-user beforehand.

IMPORTANT INFORMATION

Each laboratory is responsible for validating assay performance on their system. These User-Defined Parameters are provided as a starting point for users and should be verified with additional testing as applicable before reporting diagnostic results. ARK Diagnostics has not reviewed performance data using these settings on this analyzer.

ARK Diagnostics, Inc. manufactures the ARK Methotrexate II Assay, Calibrators, Controls, and Dilution Buffer and is solely responsible for the quality of the data obtained which is caused by performance of the reagent, any variation between lots of ARK Methotrexate II reagent, ARK Methotrexate II calibrator, ARK Methotrexate II control, or ARK Methotrexate II Dilution Buffer. ARK Diagnostics is not responsible for user-defined changes.

Roche Diagnostics does not manufacture the ARK Methotrexate II reagent or perform quality control or other tests on individual lots. Roche Diagnostics cannot be responsible for the quality of the data obtained which is caused by performance of the reagent, any variation between lots of ARK Methotrexate II reagent, ARK Methotrexate II calibrator, ARK Methotrexate II control, or ARK Methotrexate II Dilution Buffer nor changes to analyzer protocols for ARK Methotrexate II Assay.

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Analytical Parameters

Long Name	Methotrexate II
Unit 1	µmol/L
Unit 2	none
Conversion Factor	1
Sample Type	Serum/Plasma

Rerun Setting

Result outside technical limit -> Below	Off	
Result outside technical limit -> Above	On	
Technical Limit	0.030	1.300

Assay

Assay	Rate			
Time	10			
Primary Wavelength	340			
Secondary Wavelength	415			
Points	**1**	**2**	**3**	**4**
	20	25		

Reagent Volume

Reagent Type	Reagent	Dilution Volume	Mode	Mixing
R1	60	0	Dummy	14
R2	0	0		14
R3	30	0	Dummy	14

Sample Volume

Sample Type	Sample	Diluted	Diluent	Mixing
Normal	3.0			4
Decreased	7	3.0	93.0	4
Increased	3.0			4

Diluent -> Type	Diluent
Diluent -> ACN	29060
Diluent -> Dilution	10.0

Cell Wash

Wash Cells With	Basic and Acid
Higher Uncertainty	0

QC Interval

QC Interval Timeout	False
QC Interval Timeout -> Hours	1

Calibration
Changeover Settings

Lot Changeover	Full
Automatic masking if calibration failed	False
Reagent Pack Changeover	Full

Calibration Trigger

QC Violation -> Method	Cancel
Timeout -> Method	Cancel
Timeout -> Stability	14 days

Limit Values

SD Limit	0.999	
Duplicate Limit	99 %	3.3 Abs
Sensitivity Start	0	
Sensitivity End	0	
Sensitivity Limit	-9.9	9.9
S1 Abs. Limit	-3.3	3.3

Calibration Method

Curve Type	RCM1
Point	6
Weighting	0
K-factor	

RCM Weighting

1	0
2	0
3	0
4	0
5	0
6	0

Calibrators
Standards

Standard ID	Calibrator Code	Calibrator Concentration	Calibrator Volume	Diluted C. Volume	Diluent Volume
S 1		**0.000**	**3.0**	0	0
S 2		**0.050**	**3.0**	0	0
S 3		**0.150**	**3.0**	0	0
S 4		**0.350**	**3.0**	0	0
S 5		**0.650**	**3.0**	0	0
S 6		**1.300**	**3.0**	0	0

Calibrator Diluent -> Type	Diluent
Calibrator Diluent -> Code	29060
Calibrator Diluent -> Dilution Factor	10.0

Checks
Kinetics unstable check

4-8 Point	0 %
9 Point	0 %
Min. Total Rate	3.3
Min. Dif. Rate	3.3

Reaction Limit

Check	Off
Abs. Limit	0
Method	Decrease

Application Correction Factor	**A**		**B**
	1		0
Sample Index Limits	**L**	**H**	**I**
	0	0	0

R.P. Settings
c packs

c pack	
No. of Tests	**120 or 247[a]**

Position	Reagent Type	Pipetting Volume	Filling Volume	Max Volume
B	R1	**60 µl**		95.0 mL
C	R3	**30 µl**		30.0 mL

c pack	None
No. of Tests	-

Position	Reagent Type	Pipetting Volume	Filling Volume	Max Volume
B	-	-	-	95.0 mL
C	-	-	-	30.0 mL

[a] No. of tests for part number 5071-0001-00 (R1 16 mL, R2 8 mL) is 120.
No. of tests for part number 5071-0001-01 (R1 28 mL, R2 14 mL) is 247.

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Auto-Dilution Protocol

ARK Diagnostics has not validated the auto-dilution procedure on the Roche cobas c 503 analytical unit. It is the responsibility of the user to verify and validate the auto-dilution settings through appropriate testing prior to reporting any patient results.

The suggested auto-dilution procedure enables automatic dilution of patient specimens by the analyzer and requires the use of the "Diluent NaCl 9%" c-pack cassette provided by Roche Diagnostics. Two dilution levels may be configured: 1:14 and 1:50.

A 1:14 dilution can be automatically triggered for overrange samples based on the programmed instrument parameters. If a 1:50 dilution is required, it must be manually programmed by the user. Follow the instructions below to manually program and perform a 1:50 dilution.

1. Go to Routine and click on "Order Tests" tab.
2. Make sure the correct Sequence No. (Sample number/Sample ID) is selected.
3. Click on the assay name box (e.g. MTX2)
4. The assay name box will have a drop down menu that allows you to select a specific dilution. Select "1:50."
5. The assay name box with the drop down menu will now say 1:50 and have a symbol "▼" next to it.
6. Run the sample.
7. In order to check if the dilution was run correctly, go to your "Results" tab and click on the sample that was run.
8. Click the "Test Review" tab.
9. If the sample was run using the 1:50 dilution, under "Dil." there will be the number "50". If the sample was run using the automatic 1:14 dilution, there will be the "▼" symbol. If the sample was run neat with no dilutions there will be the "□" symbol.
10. For any specimens which are overrange at 1/50 dilution (that is, above about 65 µmol/L) the customer will need to perform a pre-dilution with the ARK Methotrexate II Dilution Buffer. The ARK Methotrexate II High Range control kit includes controls at 5, 50, and 500 µmol/L for verifying the accuracy of these dilutions.

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