ARK Diagnostics, Inc.

CE

ARK™ Methotrexate Assay
Roche cobas® c 503 Analytical Unit

For In Vitro Diagnostic Use

The ARK Methotrexate 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. The following are parameters for use when performing the ARK Methotrexate Assay on Roche cobas pro chemistry systems. 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 Methotrexate package inserts for reagent, calibrator, control and dilution buffer are available online at www.ark-tdm.com.

ORDERING INFORMATION

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

Product	Catalog Number
ARK Methotrexate Assay (c pack green)	5026-0001-03
ARK Methotrexate Calibrators (6 x 2 mL)	5026-0002-00
ARK Methotrexate Control (6 x 2 mL; LOW, MID, HIGH, 5, 50, 500 µmol/L)	5026-0003-00
ARK Methotrexate Control (3 x 2 mL; LOW, MID, HIGH)	5026-0003-01
ARK Methotrexate Control (3 x 2 mL; 5, 50, 500 µmol/L)	5026-0003-02
ARK Methotrexate Dilution Buffer (25 mL)	5026-0004-00

Required but not provided (Contact Roche Diagnostics)

Roche cobas c pack MULTI open/close tool

Roche cobas c pack Diluent NaCl 9% (Cat. No. 08063494190)

ROCHE C PACK GREEN CONVENIENCE PACKAGE

ARK Diagnostics provides a convenience package version of the test kit for the ARK Methotrexate Assay using catalog number 5026-0001-03 for ordering. This convenience package is provided to assure the items necessary to perform the ARK Methotrexate Assay on the cobas c 503 analytical unit. The test kit contains antibody reagent R1, enzyme reagent R2, empty cobas c pack green, and two funnels for transferring R1 and R2 respectively to the c pack green. Instructions for filling the cobas c pack green are provided below. Roche customers must obtain the "Open/Close Tool" and "Diluent NaCl 9%" separately from Roche Diagnostics.

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PREPARATION OF ASSAY COMPONENTS

The following assay components are ready-to-use liquids as supplied. 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 one of the enclosed funnels 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 one of the enclosed funnels 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 MTX 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.

Calibrator and Control information can be downloaded on to the Roche cobas c 503 analytical unit by searching for the calibrator or control codes provided below.

Calibrator Short Name	Calibrator Code
MTX 1	20601
MTX 2	20602
MTX 3	20603
MTX 4	20604
MTX 5	20605
MTX 6	20606

Control Short Name	Control Code
MTX LOW	20169
MTX MID	20170
MTX HIGH	20171
MTX 5	20172
MTX 50	20173
MTX 500	20174

NOTE: ARK Diagnostics products have lot numbers with letters WO followed by a five digit numerical number (e.g. WO12345). The Roche cobas c 503 analytical unit displays lot numbers as a six digit numerical number. Because of the difference in how the lot information is displayed on the analyzer, all lot numbers for ARK Diagnostics products will be displayed without the WO and with a leading 0. For example if an ARK Diagnostic product has lot number WO12345, the Roche cobas c 503 analytical unit will display the lot number as 012345.

SPECIMEN COLLECTION AND PREPARATION

Each assay requires serum or plasma. Whole blood cannot be used. Blood collection devices compatible with TDM should be used. The anticoagulants sodium heparin, lithium heparin and potassium EDTA have been tested for interference in the ARK Methotrexate Assay and may be used. For consistency, using the same specimen matrix for individual patients is a good practice. Store specimens as recommended. ARK Methotrexate Dilution Buffer may be used to dilute specimens with high drug concentrations if necessary. Test as recommended in the package insert.

Precaution: ARK Diagnostics has validated Roche saline as a sample diluent for use in the auto-dilution procedure. Special attention to applicable instrument programming is provided below. The use of alternate diluents and dilution protocols for specimens containing high concentrations of methotrexate should be validated by the end-user beforehand.

IMPORTANT INFORMATION

This application is based on limited technical evaluation. The evaluation is intended to provide guidance only for the use of the ARK Methotrexate Assay on the Roche cobas c 503 analytical unit on cobas pro systems. Additional information regarding expected values, warnings and precautions, procedures, calibration, quality control, limitations, and technical performance is provided in ARK Methotrexate package inserts.

Each laboratory is responsible for validating assay performance on their system. The parameters provided should be verified with additional testing as applicable before reporting diagnostic results. The Roche cobas c 503 analytical unit is available on cobas pro systems.

Roche Diagnostics does not manufacture the ARK Methotrexate 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 reagent, ARK Methotrexate calibrator or

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ARK Methotrexate control nor changes to analyzer protocols for the ARK Methotrexate Assay.

INSTRUMENT SETTINGS

Application

Analytical Parameters ▼

ACN:	21440	☑ Automatic Rerun		Assay:	Rate	Wavelength	
Unit:	µmol/L			Time:	10	Primary:	340
		Result Outside Technical Limit		Points		Secondary:	415
		Below:	Off	1:	20	2: 25	
		Above:	On	3:	0	4: 0	
Technical Limit:		0.0400 -	1.20				
Repeat Limit:		-99999 -	999999				

Reagent Volume

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

R.P. Settings

Special Reagent
Used: No
ACN:
Timing:
Reagent Volume:
Dilution Volume:

Sample Volume

	Sample	Diluted	Diluent	Mixing
Normal:	3.4	0.0	0	4
Decreased:	10.0	3.4	90	4
Increased:	3.4	0.0	0	4

Diluent
Type: Diluent
ACN: 29060
Dilution: 10.0

Wash Cells with: Detergent1
Higher Uncertainty: 0.0400

☐ QC Interval Timeout 1 Hours

R.P. Settings

Pattern 1 ▼

System ID:

Position	Reagent Type	Bottle Type	No. of Tests	Volume	
B	R1	L	140	14.5	mL
C	R3	S	140	8.8	mL

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Calib. ▼

Changeover Settings

Lot Changeover	Full	☑ Automatic Masking if calibration has failed
Reagent Pack Changeover	Off	

Other Settings

⦿ Timeout
○ QC Violation

QC Violation Method

Rule

Timeout Type	Method	Stability		QC
Lot:	Cancel	0	Days	QC 1
R.P.	Cancel	0	Days	QC 2
				QC 3

Limit Values

SD Limit:	0		
Duplicate Limit:	10	%	
	0.0200	Abs.	
Sensitivity Start:	1		
Sensitivity End:	5		
Sensitivity Limit:	-99	-	99
S1 Abs. Limit:	-3.3000	-	3.3000

Calibration Method

Curve Type:	RCM1	Point:	6	Weighting	3
RCM Weighting		Update Type			
1:	2	1 Point A:			
2:	8	1 Point B:			
3:	8	2 Point:			
4:	1	Update Point:			
5:	1	1 Point A:	1	2 Point 1:	1
6:	1	1 Point B:	1	2 Point 2:	1

Calibrators ▼

	S1	S2	S3	S4	S5	S6
Calibrator Code:	20601	20602	20603	20604	20605	20606
Concentration:	0.0000	0.0500	0.1500	0.2500	0.5000	1.200
Rack ID – Position:						
Calibrator Volume:	3.4	3.4	3.4	3.4	3.4	3.4
Diluted C. Volume:	0	0	0	0	0	0
Diluent Volume:	0	0	0	0	0	0

Calibrator Diluent

Type:	Diluent
Code:	29060
Dilution Factor:	10.0

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Ranges ▼				
Linearity Limit			High Dose Hook Effect	
4-8 Point:	0	%	Point1:	1
9 Point:	0	%	Point2:	1
Min. Total Rate:	0		Factor:	0.0000
Min. Diff. Rate	0		Offset:	0.0000
Reaction Limit				
Check:	OFF			
Abs. Limit:	0		Method:	Decrease
Abs. Point1:	1		Abs. Point2:	1
Sample Blank Point:	1			
Reduction Point:	4			
Blank Correction:	High			
Application Correction Factor			Sample Index Limits	
A:	1		L:	0
B:	0.000		H:	0
			I:	0

Auto-Dilution Protocol

ARK Diagnostics has validated "Roche saline" as a sample diluent for use in the auto-dilution procedure on the Roche cobas c 503 analytical unit. The auto-dilution procedure allows for automatic dilution of the patient specimen by the analyzer and requires the use of the "Diluent NaCl 9%" c-pack cassette supplied by Roche Diagnostics. Two levels of dilution are possible. ARK validated the use of 1/10 and 1/50 dilutions.

A 1:10 dilution will automatically be performed according to the specified instrument parameters for overrange samples. If a 1:50 dilution is needed, it will have to be programmed manually. Follow the steps below in order to manually program and run 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. MTX)
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 1:10 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 60 µmol/L) the customer will need to perform a pre-dilution with the ARK Methotrexate Dilution Buffer. The ARK Methotrexate High Range control kit includes controls at 5, 50, and 500 µmol/L for verifying the accuracy of these dilutions.

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