

MGB Alert[®] CRE RUO Mix



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For Research Use Only. Not for use in diagnostic procedures.

REF
M800692

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Intended Use

The **MGB Alert CRE RUO Mix** is intended for use in laboratory developed tests which target gene markers of carbapenem-resistant enterobacteriaceae (CRE) using real-time PCR. The assay requires trained personnel, skilled in molecular biology techniques.

Product Description

The **MGB Alert CRE RUO Mix** package includes a 20x mix of primers and probes which, combined with master mix as a monoreagent, amplify and detect the CRE genetic markers and Internal Control as shown in the table below. The majority of target-specific probes are labelled with the AquaPhluor[®] (AP $_{nnn}$) family of dyes, where nnn designates the approximate absorbance maximum. The probe chemistry employed is DSQ-TaqMan, the next generation of MGB-TaqMan, which generates a fluorescent signal due to enzymatic hydrolysis of the fluorophore when the probe is hybridized to its specific amplicon.

Gene Marker (or Internal Control)	Fluorophore	InGenius Channel	Control Part Number
<i>bla</i> _{KPC}	FAM	1	M400668 (250 μL)
<i>bla</i> _{IMP}	AP525	2	M400669 (250 μL)
<i>bla</i> _{OXA}	AP559	3	M400670 (250 μL)
<i>bla</i> _{NDM}	AP593	4	M400671 (250 μL)
<i>bla</i> _{VIM}	AP639	5	M400672 (250 μL)
Internal Control 2	AP690	6	M400276 (35 μL) M400398 (250 μL)

The MGB Alert CRE RUO Mix is provided at a volume of 125 μL at a 20x concentration relative to the final PCR. The controls listed in the table above are recommended, but not provided with the MGB Alert CRE RUO Mix. Additional recommended materials which are not included are listed in the following table.

EG Part Number	Material	Use
M800599 (300 µL) M800691 (3 mL)	MGB Alert Platinum Master Mix	Contains polymerase with exonuclease activity, buffers, dNTPs, and excipients for PCR at 8x final concentration
M600053	MGB Alert CRE RUO Assay Parameters	Template for PCR parameters, Ct cutoffs, and interpretation model parameters
M800538	ELITE InGenius Instrument	Sample to result extraction and PCR instrument with parallel processing of 12 samples
M800530	ELITE InGenius Extraction Cassette	Extraction cassette for processing 200 µL samples (48) on ELITE InGenius
M800536	ELITE InGenius Consumable Set	Tips, sonication tubes and caps, and microtubes for ELITE InGenius extraction (48)
M800537	ELITE InGenius PCR Cassettes	Specialized reaction vessels specific for ELITE InGenius (192)
NA	PCR grade water	Monoreagent preparation, negative controls
NA	TE-TYR buffer	Tris-EDTA buffer (1X, pH 8.0) with total yeast RNA (0.1 mg/mL) to dilute and preserve control templates

It is recommended to protect all reagents from light, store at ≤ -10 °C while not in use, and limit the number of freeze-thaws of all reagents to 10 or fewer.

Control Setup

It is recommended to prepare stock dilutions of the highly concentrated positive controls (PC). Mixtures of the diluted controls, in two sets, may be used on ELITE InGenius® to automatically produce control plots. The recommended dilution scheme is shown in the table below and the recommended diluent is TE-TYR buffer. Aliquots (25-500 µL) of the final PC Set 1 and PC Set 2 may be prepared in 0.5 mL skirted microtubes with screw caps, provided with the ELITE InGenius Consumable Set.

PC	Intermediate Dilutions to 1000 copy/µL	Final Combination, PC Set 1
KPC (M400668), ~1e7 copy/µL	2 @ 100x serial dilutions	Combine 25 µL each 1000 copy/µL template with 925 µL diluent to produce 1 mL PC Set 1 at ~25 copy/µL
OXA (M400670), ~1e7 copy/µL	2 @ 100x serial dilutions	
VIM (M400672), ~1e7 copy/µL	2 @ 100x serial dilutions	

PC	Intermediate Dilutions to 1000 copy/µL	Final Combination, PC Set 2
IMP (M400669), ~1e7 copy/µL	2 @ 100x serial dilutions	Combine 25 µL each 1000 copy/µL template with 925 µL diluent to produce 1 mL PC Set 2 at ~25 copy/µL
NDM (M400671), ~1e7 copy/µL	2 @ 100x serial dilutions	
IC2 (eg, M400276), ~1e6 copy/µL	3 @ 10x serial dilutions	

The recommended concentration of IC2 template for extraction is 1000 copy/µL.

Reaction Setup

Refer to the ELITE InGenius Manual or Quick Start Guide to set up the InGenius instrument. The MGB Alert CRE RUO Assay Parameters may be obtained from Technical Assistance.

The InGenius extraction protocol for MGB Alert CRE RUO uses 200 µL sample volume and elutes the purified nucleic acids into 100 µL water. The sample may either be used from a compatible primary tube or manually transferred to a sonication tube. (Note: sonication is not employed in the MGB Alert CRE RUO Assay Parameters.) For the Internal Control 2 template (1000 copy/µL), it is required to:

- Prepare at least $12n + 12$ µL per extraction (eg, prepare $(12)(3) + 12 = 48$ µL for three extractions)
- Prepare the IC2 template in a 0.5 mL microtube for InGenius
- Carefully spin down the IC2 template before placing in the InGenius to ensure accurate pipetting

The InGenius assay parameters specify a 40 µL reaction using 20 µL monoreagent with 20 µL sample. For the 2x monoreagent, it is required to:

- Prepare at least $24n$ µL of 2x monoreagent per PCR (eg, prepare $(24)(3) = 72$ µL for three PCR)
- Prepare the monoreagent in a 2 mL microtube for InGenius
- Carefully spin down the monoreagent before placing in the InGenius to ensure accurate pipetting

Component	1 reaction*	12 reactions*
water	15.6 µL	187.2 µL
Platinum Master Mix, 8x	6.0 µL	72.0 µL
CRE RUO Mix, 20x	2.4 µL	28.8 µL
Total volume	24.0 µL	288 µL

* Total volume includes dead volume for InGenius

The MGB Alert CRE RUO Assay Parameters include default settings for extraction parameters, PCR cycling parameters, Ct cutoff, control criteria, data analysis, and interpretation models. Performance of the assay may require modification depending on sample type—please contact Technical Assistance for guidance on modifying Assay Parameters.

The MGB Alert CRE RUO Assay Parameters include an Assay Specific Crosstalk Compensation Matrix for this particular probe set. Slight variations in InGenius instruments may require some modification of the Crosstalk Matrix—please contact Technical Assistance for guidance on modifying the Crosstalk Matrix.

Technical Assistance

For Technical Assistance, please contact:

- Toll free 1-800-453-2725 (options for Molecular Diagnostics)
- MDx@elitechgroup.com

Symbols



Catalog number



Contents



Batch code



Upper storage temperature



Date of manufacture



Use by



Manufacturer



Consult instructions for use

Legal Notices

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MGB Alert detection reagents are covered by one or more of US Patents Numbers 6949367, 6962991, 6972339, 7045610, 7112684, 7319022, 7348146, 7381818, 7541454, 7582739, 7601851, 7662942, 7671218, 7718374, 7723038, 7759126, 7767834, 7851606, 8008522, 8067177, 8163910, 8389745, 8569516, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, and 10738346 as well as applications that are currently pending.

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