



Antibiotic Resistance RT-qPCR

Recommended User Guide

For Research Use Only. Not for Use in Diagnostic Procedures.

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1.1 Kit Contents & Materials

Item	SKU	QTY	Volume	Included
PureAmp Mastermix	N/A	1	5mL or 20mL	Yes
Modules 1-4	EX-ABR-250(1000)	1	250µL or 1000µL	Yes
ABR Amplification Control	CTRL-ABR	-	1mL	No
FAM	FAM-96(384)	-	-	No
SUN	SUN-96(384)	-	-	No
Texas Red	TxR-96(384)	-	-	No
CY5	CY5-96(384)	-	-	No
TAMRA	TAMRA-96(384)	-	-	No

1.2 Contents Storage

- i. **Mastermix:** Upon receipt, store at -20 to -80°C in a constant temperature (non-frost free, manual defrost) freezer.
- ii. **Assay (Modules):** Upon receipt, store at -15 to -30°C in a constant temperature (non-frost free, manual defrost) freezer. Minimize free thawing and exposure to light. Modules can be stored in refrigerator temperatures for short term use.
- iii. **ABR Amplification Control:** Upon receipt, store at -15 to -30°C in a constant temperature (non-frost free, manual defrost) freezer. Minimize free thawing and exposure to light. It is recommended to dilute control upon receipt and create multiple aliquots to avoid multiple freeze thaws. Control is supplied in 1mL aliquots at 5×10^5 copies/µL.

2.1 Modules

- This kit consists of 4 modules containing the following primer/probe sets:

Module 1	Probe
Sul 1, Sul 2	FAM
dfrA1, dfrA5	SUN
qnrA, qnrB	TXR
IMP-1, IMP-2	CY5
Bacillus atrophaeus	TAMRA
Module 2	Probe
ErmA, ErmB, ErmC	FAM
TetM	SUN
blaSHV	TXR
CTX-M1/2/9/8	CY5
Bacillus atrophaeus	TAMRA
Module 3	Probe
OXA-23, OXA-48, OXA-58	FAM
KPC-3	SUN
NDM-1	TXR
VIM-1, VIM-2	CY5
Bacillus atrophaeus	TAMRA
Module 4	Probe
vanA, vanB, vanC	FAM
mecA, mecB, mecC	SUN
Ampicillin	TXR
OXA-1	CY5
Bacillus atrophaeus	TAMRA

3.1 Run Parameters

- Block Type: 384
- Standard Curve
- Taqman Reagents
- Passive Reference: None

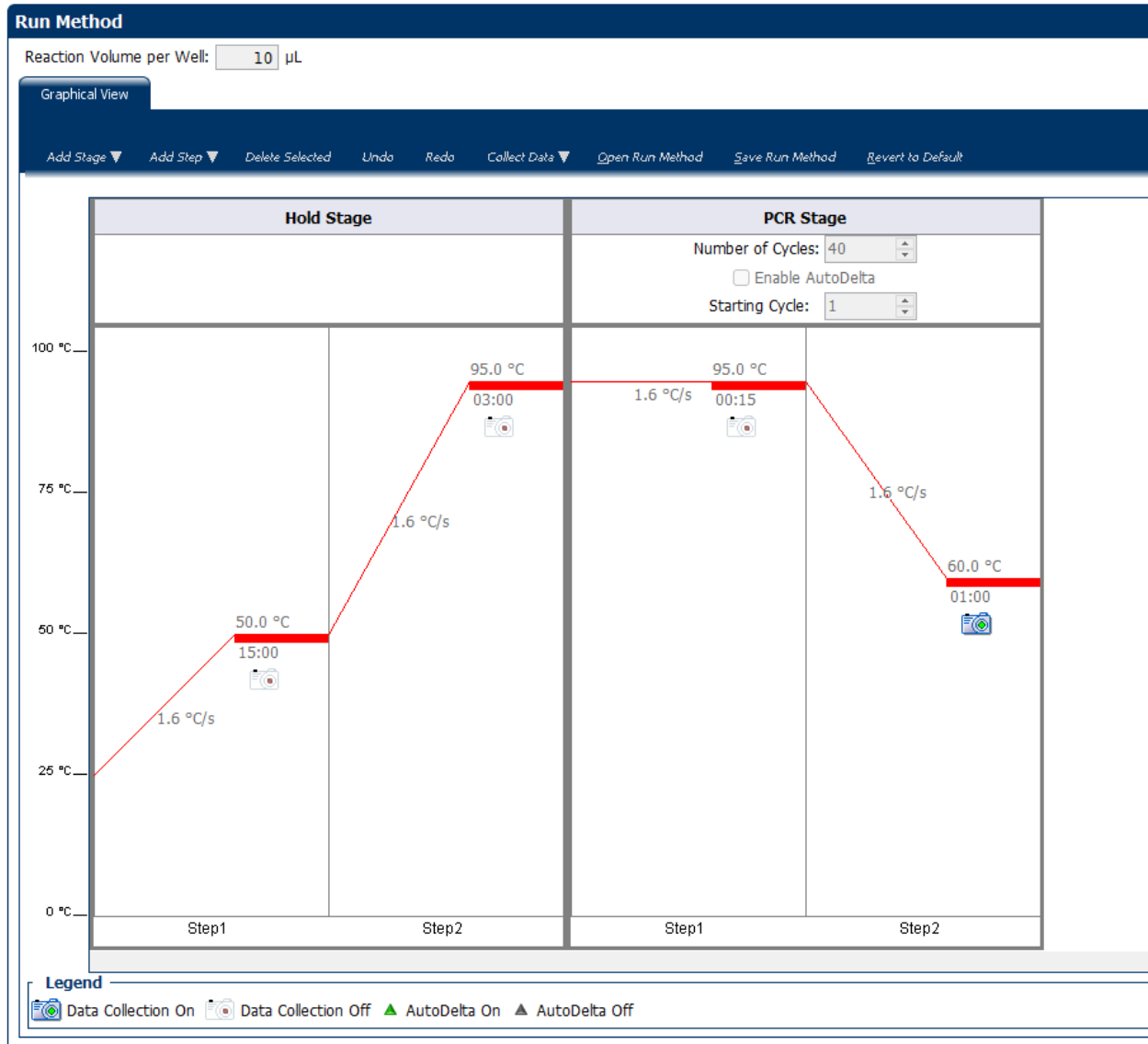
3.2 Instrument Calibration

The following fluorophores are used to detect amplification:

- FAM
- SUN (VIC)
- Texas Red
- CY5
- TAMRA

Please ensure your instrument is calibrated for these fluorophores prior to proceeding. If you are missing any of these, calibration plates can be purchased directly from www.A2KScientific.com with next day shipping.

3.3 Run Method



4.1 PCR Setup

- i. Remove kit components from storage and thaw. Once thawed, modules should be vortexed and centrifuged. Mastermix should be mixed gently by inversion or gentle vortex.
- ii. In a nuclease free container, mix the following according to the total number of samples. Please note that 10-15% overage may be required to account for volume loss when pipetting. The following mix is for a single module:

Component	Volume/Sample
PureAmp Mastermix	5 μ L
Module	1 μ L

- iii. After sufficient vortex and centrifuge, 6 μ L of reaction mix should be added to each well followed by 4 μ L of purified nucleic acid (sample or control) to each well of a 384 well optical PCR plate.
- iv. Once desired modules have been added to the plate along with purified nucleic acid, the plate should be sealed, vortex, and centrifuged.
- v. Place plate in instrument and start run.