# AriaMx HRM Calibration Plate Kit

## Part Number 5190-7702

## Protocol

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

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Agilent Technologies

# Notices



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# In this Guide...

This protocol provides instructions for using the AriaMx HRM Calibration Plate Kit.

## What's New in Version B0

- Updated contact information for Agilent Technical Support.

# Content

# 1 Before You Begin



Make sure that you read and understand the information in this chapter before you start an experiment.

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## Overview

The AriaMx Real-Time PCR System allows you to perform high resolution melt (HRM) analysis. HRM analysis is a technique used for genotyping samples that include a single nucleotide polymorphism (SNP) in the DNA sequence. Applications that may use HRM analysis include species identification, mutation screening, and haplotype characterization.

In order to use HRM analysis on the AriaMx instrument, you must run an HRM calibration experiment using an HRM calibration plate (HCP). The AriaMx HRM Calibration Plate Kit contains a 96-well plate for use in an HRM calibration experiment. The plate is pre-aliquoted with a master mix containing a DNA product and the double-stranded DNA-binding dye EvaGreen.

## Kit components

The AriaMx HRM Calibration Plate Kit contains one 96-well plate. Each of the 96 wells contains 20 µL of pre-aliquoted reagents.

## Plate storage and handling

The plate is shipped on dry ice. Store at -20°C upon receipt.

Wear chemical-resistant powder-free gloves whenever handling the HRM Calibration Plate.

# 2 Protocol



This chapter provides instructions on running an HRM calibration experiment using the AriaMx HRM Calibration Plate.

## Prepare the plate

Prepare the plate just before starting an HRM calibration experiment.

1. Thaw the AriaMx HRM Calibration Plate at room temperature for 5-15 minutes. *Do not remove the seal.*
2. Once thawed, mix the contents of the wells by completing the following actions five times.
   a. Invert the plate so that the liquid moves to the seal on top of each well.
   b. Re-invert the plate to return the liquid to the bottom of each well.
3. After completing five mixing cycles, briefly spin the plate in a plate centrifuge at 500 × g for at least one minute.
4. Verify that no bubbles are present at the bottoms of the wells, beneath the liquid.

   While it is critical to remove any bubbles from the bottom of the tubes, small bubbles near the liquid meniscus should not affect the test results.

## Run the HRM calibration experiment

You must set up the HRM calibration experiment directly on the instrument (you cannot set up an HRM calibration experiment from the AriaMx software on your PC).

Verify that the FAM/SYBR optical module is installed in the AriaMx instrument before you begin.

1. Load the AriaMx HRM Calibration Plate into the AriaMx instrument and close the instrument door.
2. On the instrument Home screen, press the HRM Calibration icon.
3. On the subsequent screen, press **Open Default Experiment**.

   The default HRM calibration experiment opens to the Plate Setup screen. All wells are set to the Unknown well type and the SYBR dye is selected for target detection in all wells.

   The EvaGreen dye used in the AriaMx HRM Calibration Plate is detectable with the FAM/SYBR optical module.

4 Navigate to the Thermal Profile screen and press **Run Experiment**.

A message box opens on the touchscreen prompting you to save the experiment. Press **OK** in the message box to save the experiment to the HCP folder.

5 Enter a file name for the experiment and press **Save**.

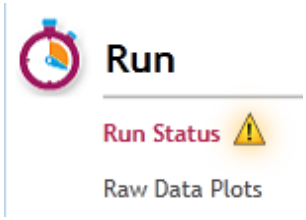
The instrument starts running the experiment.

6 After the run, a message box opens on the screen notifying you if the calibration passed or failed.

- If it passed, copy the post-run experiment file to your PC to be used for calibration in experiments with an HRM segment.
- If it failed, see "If the HRM calibration experiment fails", below.

## If the HRM calibration experiment fails

If the calibration experiment failed the system's quality check, the touchscreen displays a message box at the end of the run notifying you of the failure. You will also see a warning icon (as shown below) if you open the experiment in the AriaMx program on your PC.




Possible causes of a failed experiment include improper handling of the plate and the presence of bubbles in the bottoms of the plate wells. Review "Plate storage and handling" on page 9 and "Prepare the plate" on page 12. If problems persist, contact Agilent Technical Support.

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## In This Book

This guide contains information to run the AriaMx HRM Calibration Plate Kit protocol.



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