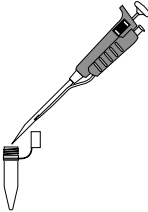
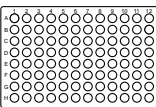
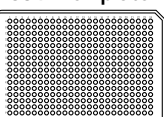
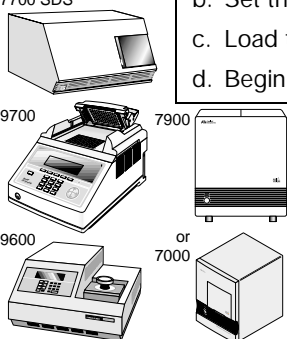
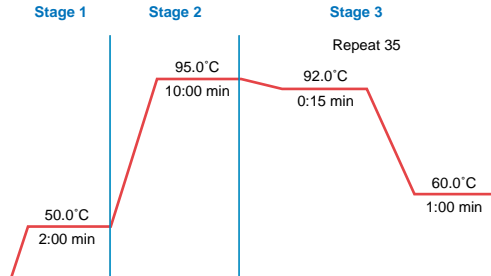


Pre-Developed TaqMan® Assay Reagents for Allelic Discrimination

Quick Reference Card

PCR Amplification

STEP	ACTION																															
1	<p>Prepare the reaction mix.</p> 	<p>For safety guidelines, please see the "Safety" section in the <i>Pre-Developed TaqMan® Assay Reagents Allelic Discrimination Protocol</i>, P/N 4312214. For all chemicals in bold type below, please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</p> <p>a. Calculate the number of reactions to be performed for each assay.</p> <p>Note: Add extra reactions to provide excess volume for the loss that occurs during reagent transfers.</p> <p>b. Using the table below, calculate the volume of Master Mix components—without sample—needed for each assay.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Component</th> <th>96-well Volume (25 µL) / Reaction</th> <th>384-well Volume (5 µL) / Reaction</th> </tr> </thead> <tbody> <tr> <td>2X TaqMan® Universal PCR Master Mix</td> <td>12.5</td> <td>2.5</td> </tr> <tr> <td>10X AD Assay Mix</td> <td>2.5</td> <td>0.5</td> </tr> <tr> <td>DNase-free H₂O</td> <td>5.0</td> <td>0.0</td> </tr> <tr> <td>Total</td> <td>20.0</td> <td>3.0</td> </tr> </tbody> </table> <p>c. Pipette the reagents into a sterile tube.</p>	Component	96-well Volume (25 µL) / Reaction	384-well Volume (5 µL) / Reaction	2X TaqMan® Universal PCR Master Mix	12.5	2.5	10X AD Assay Mix	2.5	0.5	DNase-free H₂O	5.0	0.0	Total	20.0	3.0															
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2	<p>Prepare the reaction plate.</p> <p>96-well plate</p>  <p>384-well plate</p> 	<p>a. As an example, you can pipette one of the controls or samples below into individual wells on an optical 96-well or 384-well plate.</p> <p>Note: Pipetting low volumes may require use of liquid handling robotics.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Sample Type</th> <th>Component</th> <th>96-well Volume (µL)</th> <th>Wells</th> <th>384-well Volume (µL)</th> <th>Wells</th> </tr> </thead> <tbody> <tr> <td>NTC</td> <td>1X TE Buffer</td> <td>5</td> <td>A1-A4</td> <td>2</td> <td>A1-A8</td> </tr> <tr> <td>AL1</td> <td>5X Allele 1 Control</td> <td>5</td> <td>A5-A8</td> <td>2</td> <td>A9-A16</td> </tr> <tr> <td>AL2</td> <td>5X Allele 2 Control</td> <td>5</td> <td>A9-A12</td> <td>2</td> <td>A17-A24</td> </tr> <tr> <td>UNKN</td> <td>Genomic DNA (2 to 20 ng/µL)</td> <td>5</td> <td>B1-H12</td> <td>2</td> <td>B1-P24</td> </tr> </tbody> </table> <p>b. Pipette 20 µL of reaction mix into each well for a 96-well plate or 3 µL of reaction mix into each well of a 384-well plate.</p> <p>c. Seal or cap the plate.</p>	Sample Type	Component	96-well Volume (µL)	Wells	384-well Volume (µL)	Wells	NTC	1X TE Buffer	5	A1-A4	2	A1-A8	AL1	5X Allele 1 Control	5	A5-A8	2	A9-A16	AL2	5X Allele 2 Control	5	A9-A12	2	A17-A24	UNKN	Genomic DNA (2 to 20 ng/µL)	5	B1-H12	2	B1-P24
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3	<p>Perform PCR.</p> 	<p>a. Program your thermal cycler with the PCR conditions.</p> <p>b. Set the reaction volume to 25 µL for the 96-well plate, or to 5 µL for the 384-well plate.</p> <p>c. Load the reaction plate into the thermal cycler.</p> <p>d. Begin thermal cycling.</p> <p>IMPORTANT! These conditions are optimized for use with TaqMan PDARs for AD only.</p> 																														

Visit our Web site for a list of available assays and consumable supplies:

www.appliedbiosystems.com/products/

Click:

[Reagents > TaqMan® Pre-Developed Assay Reagents for Allelic Discrimination](#)

or visit the Applied Biosystems Store: <http://store.appliedbiosystems.com/>

In the US:

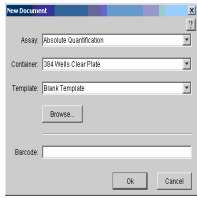
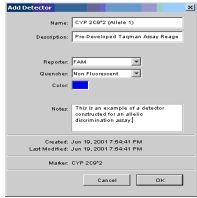
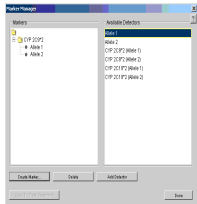
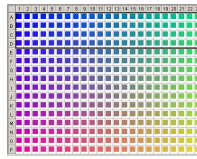
Call Applied Biosystems toll-free at 1.800.345.5224.

Outside the US:

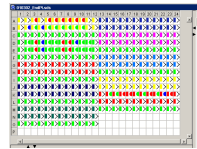
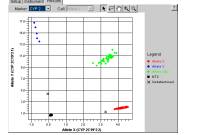

Use the Internet for the location of your local Applied Biosystems sales representative.

<http://www.appliedbiosystems.com>

Allelic Discrimination Plate Read

STEP	ACTION	
1	<p>Set up new plate read file.</p> 	<ol style="list-style-type: none"> Launch the SDS software on the 7900HT SDS computer. (If using the 7700 or 7000 instrument, refer to the specific user manual for each instrument.) In the New Document window, create a new plate read file with the following attributes. <ul style="list-style-type: none"> Under the Assay menu, select Allelic Discrimination. Under the Container menu, select the plate type (96-well or 384-well). Under the Template menu, select Blank Template. Click OK. A new plate document opens with the appropriate attributes.
2	<p>Create new markers.</p> 	<ol style="list-style-type: none"> Open Detector Manager from the Tools menu. Click File/New to open the Add Detector dialog box and fill in the following information. <ul style="list-style-type: none"> Type the name of Allele 1, select FAM for reporter, and Non-Fluorescent for quencher. Type the name of Allele 2, select VIC for reporter, and Non-Fluorescent for quencher. Click Done. The Add Detector dialog box closes. In the Tools menu, select Marker Manager and click Create Marker. The Add Marker dialog box opens. Click the Enter name of new marker text field, type the new name, and click OK.
3	<p>Apply detectors to the new marker.</p> 	<p>IMPORTANT! A marker must be configured with two detectors before it can be applied to a plate document.</p> <ol style="list-style-type: none"> In the Markers text field, select the new marker. In the Available Detectors text field, click the detector (allele 1) you want to add to the marker. Click Add Detector. Repeat steps a through c for the second detector (allele 2), and click Done.
4	<p>Select the sample wells and read the plate.</p> 	<p>Select wells:</p> <ol style="list-style-type: none"> Select the wells in the grid pane that contain your samples. Add the marker to those selected wells by checking the Use box adjacent to the desired marker in the Set Up window, and repeat for other wells. <p>Read plate:</p> <ol style="list-style-type: none"> Save the document, and select the Instrument tab. Place the thermal cycled plate in the instrument, and select Plate Read on the Instrument page. When the plate read has finished, save the file.

Allelic Discrimination Analysis

STEP	ACTION	
1	<p>Open the plate document file.</p> 	<ol style="list-style-type: none"> If the plate document file is not open, select Open in the File menu. In the Look in text field, navigate to and select the plate document file. Click Open. The SDS software displays the plate document file. In the Analysis menu, select Analyze.
2	<p>Call the allele types.</p> 	<ol style="list-style-type: none"> Click the Results tab. The software displays the Allelic Discrimination Plot. Use the lasso tool to select one cluster of data points.  In the Call drop-down list, select the appropriate call and repeat for remaining clusters. Select File/Export and export the results table.

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

Refer to the *Pre-Developed TaqMan® Assay Reagents for Allelic Discrimination Protocol* for further patent and trademark statements. Please refer to the Safety section of the protocol for safety guidelines.