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I. Description

The Plexor™ qPCR and qRT-PCR Systems^(a,b,c) are compatible with a variety of real-time PCR instruments. Data from these instruments can be analyzed with one dedicated software program, the Plexor™ Analysis Software. This manual includes instructions and thermal cycling conditions specific for use of the Plexor™ qPCR System, Plexor™ One-Step qRT-PCR System and Plexor™ Two-Step qRT-PCR System with the Roche LightCycler® 480 System. Instructions are included for instrument setup, data transfer from the instrument to the Plexor™ Analysis Software and data analysis.

II. Plate Preparation and Amplification

Detailed instructions describing assay setup are provided in the *Plexor™ qPCR System Technical Manual #TM262*, *Plexor™ One-Step qRT-PCR System Technical Manual #TM263* or *Plexor™ Two-Step qRT-PCR System Technical Manual #TM264*.

When using the Plexor™ qPCR System for the first time, we recommend programming the thermal cycling conditions and checking that the instrument is compatible with the dyes used and is configured for those dyes before assembling the reactions, so the reactions are not kept on ice for prolonged periods of time. Once you are familiar with the programming process, the instrument can be programmed after reaction assembly.

Materials to Be Supplied By the User

- LightCycler® 480 multiwell reaction plate
 - centrifuge compatible with a 96-well plate
 - optical adhesive covers and applicator
1. After the amplification reactions have been assembled, cover the reaction plate with an optical adhesive cover using the applicator. Centrifuge briefly to collect contents at the bottom of each well.
Note: Keep the plate on ice during reaction setup and programming of the thermal cycling conditions.
 2. Program the Roche LightCycler® 480 System. The proper thermal cycling conditions and instructions for programming the instrument are provided in Section III (generating color compensation files), Section IV (qPCR and two-step qRT-PCR assays), Section V (one-step qRT-PCR assays) and Section VI (genotyping assays).