

Bio-Rad CFX Connect Real Time PCR System protocol
w/SYBR green chemistry

Reaction Mixture: for use in 96 well plates, reaction volume 20 uL

Reagent	Volume (uL)
cDNA* _____	5
1.25 uM Primer pair* _____	5
10 X Master Mix _____	10.0
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Total _____	20.0

(* = concentrations /volumes can be altered for optimization. I use cDNA made from iScript kit starting from 1ug RNA, then dilute the 20ul reaction to 195 ul with water after the reaction is done.)

1. Pipette diluted cDNA into wells. When finished, tap down plate so drops fall to bottom of wells.
2. Pipette primer pair (@ 2.5 uM) into wells. When finished, tap down plate so drops fall to bottom of wells.
3. Pipette master mix into wells. Tap down.
4. Apply adhesive cover. Gently vortex plate in plate rack for 5 s. Spin 5 min @1500 x g.
5. Open lid of CFX Connect and carefully place plate in machine with well A1 in upper left corner. Push button to close lid.

Operating CFX Connect Real Time PCR machine:

1. Be sure machine is on.
2. Open CFX Manager Software.
3. Click **File → New → Run**
4. Choose protocol file by clicking “Create new,” “Select Existing,” or choose a protocol from the Express Load files. Following is the protocol I use:
 - a. 1. 95C for 30s
 - b. 2. 95C for 5s
 - c. 3. 57C for 30S + plate read
 - d. 4. GOTO 2 39 times
 - e. End
5. Click **Next**. Now you are on the plate tab. Choose a plate file by clicking “Create New,” “Select Existing,” or choose a file from Express Load. Be sure you have selected either All Channels or SYBR as your fluorophore.
6. Click **Next**. Now you are on the Start Run tab. Check the box next to the machine that your plate is in. Be sure to type the correct sample volume.
7. Click **Start Run**.
8. Save file in your folder.
9. Now you can edit the plate document if you wish. Click on **Real Time Status → View/Edit Plate** and edit as appropriate. Don't forget to save!

Product #s

Bio-Rad SsoAdvanced SYBR Green SuperMix , #172-5260

Bio-Rad Low 96-well Clear reaction plates, #MLL9601

Bio-Rad Microseal 'B' Film, #MJ0558

Bio-Rad iScript cDNA Synthesis Kit, #170-8891