Type-it® CNV Probe PCR+ qC Kit

The Type-it CNV Probe PCR+ qC Kit (cat. nos. 206662 and 206664), including buffers and reagents, should be stored immediately upon receipt at –20°C in a constant-temperature freezer and should be protected from exposure to light. The 2x Type-it Probe PCR Master Mix should also be protected from exposure to light and can be stored at 2–8°C for up to 1 month (depending on the expiration date), without showing any reduction in performance.

For more information, please refer to the *Type-it CNV Probe PCR+ qC Kit Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- This protocol is optimized for relative quantification ($\Delta\Delta C_T$) of DNA copy number in the human genome and is intended for use with the Type-it CNV Probe PCR+ qC Kit and TaqMan® probes on all real-time cyclers, including the Rotor-Gene® Q, and all instruments from Applied Biosystems, Bio-Rad, Cepheid, Eppendorf, Roche, and Agilent. Using this protocol, duplex or multiplex PCR is carried out in the presence of ROX™ passive reference dye.
- For each gene of interest (GOI), we recommend using the provided Type-it CNV Reference Probe Assay in the same reaction well as a universal reference assay for reliable $\Delta\Delta C_T$-based quantification of the CNV in the human genome. The TaqMan probe of the reference assay is labeled with MAX™ dye, which is detected on the HEX™/VIC® channel.
- We recommend preparing a 25x primer–probe mix containing specific primers and probe (recommended concentrations in the 25x primer–probe mix: 10 µM of each primer and 5 µM of the probe) for each GOI. See Tables 1 and 3 for details.
- It is recommended to use a FAM™ labeled probe to detect the gene of interest by duplex PCR on any cycler. For multiplex analyses, we strongly recommend using dual-labeled probes with nonfluorescent quenchers (standard TaqMan or TaqMan MGB™ Probes). For information on suitable combinations of reporter dyes for triplex or 4-plex PCR on various cyclers, please refer to *Type-it CNV*

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Probe PCR+ qC Kit Handbook, which can be found at www.qiagen.com/handbooks.

- To reconstitute the Type-it CNV Reference Probe Assay (100) to a 25x working solution, briefly centrifuge the tube, add 110 µl Buffer TE (pH 8.0; provided with the kit), and mix by vortexing the tube 4–6 times. If necessary, gently warm the tube to help the primers dissolve.
- Always use 30 pg–30 ng template DNA (see Tables 1–3) and the optimized cycling conditions specified in Table 4.
- The PCR must start with an initial incubation step of 5 min at 95°C to activate HotStarTaq® *Plus* DNA Polymerase.

1. Thaw the Type-it Probe PCR Master Mix, template DNA, Type-it CNV Reference Probe Assay 25x working solution, 25x primer–probe mix containing primers and probe for the gene of interest, and RNase-free water. Mix the individual solutions and place them on ice.
2. Prepare a reaction mixture according to Tables 1 or 2 for duplex PCR and Table 3 for triplex and 4-plex PCR.
3. Mix the reaction mixture thoroughly and dispense appropriate volumes into PCR tubes or the wells of a PCR plate or Rotor-Disc®.
4. Add template DNA (start with 10 ng; use 30 pg–30 ng as the range) to the individual PCR tubes or wells. See Tables 1–3 for details.
5. Program the real-time cycler according to Table 4.

   Note: For correct instrument setup for multiplex analysis (e.g., setting up detection of multiple dyes from the same well), refer to the user manual for the real-time cycler and follow the manufacturer's instructions. Be sure to activate the detector for each reporter dye used. Depending on your instrument, it may also be necessary to perform a calibration procedure for each of the reporter dyes before they are used for the first time.
6. Place the PCR tubes, Rotor-Disc, or plate in the real-time cycler, and start the cycling program.
7. Perform data analysis.

   Note: Before performing data analysis, select the analysis settings for each probe (i.e., baseline settings and threshold values). Optimal analysis settings are a prerequisite for accurate quantification data.

   Note: Only if using the Applied Biosystems® 7500, 7500 Fast, or ViiA™ 7 Real-Time PCR Systems, it is recommended to use the 'manual C_T' function instead of the 'auto C_T' function for data analysis. Use a value of 0.01 as a

starting point for the threshold setting. For all other cyclers, use the automatic C_T function as a starting point.

Table 1. Reaction setup for duplex PCR

Component	Volume (µl)
Reaction mix 2x Type-it Probe PCR Master Mix	12.5
25x Type-it CNV Reference Probe Assay solution	1
25x primer–probe mix for GOI* (FAM labeled)	1
RNase-free water	Variable
Template DNA (added at step 4)	Start with 10 ng (use 30 pg–30 ng as the range)
Total reaction volume	**25**†

* **IMPORANT**: For duplex PCR, a 25x primer–probe mix consists of 10 µM forward primer, 10 µM reverse primer, and 5 µM probe in Buffer TE, resulting in a final concentration of 0.4 µM forward and reverse primer and 0.2 µM probe.

† If the real-time cycler requires a final reaction volume other than 25 µl, adjust the amount of master mix and all other reaction components accordingly. If using 384-well plates on the ABI PRISM® 7900, use a reaction volume of 10 µl.

Table 2. Reaction setup for duplex PCR using TaqMan Copy Number Assays from Life Technologies‡

Component	Volume (µl)
Reaction mix 2x Type-it Probe PCR Master Mix	12.5
25x Type-it CNV Reference Probe Assay solution	1
20x TaqMan Copy Number Assay for GOI (FAM labeled)‡	1.25
RNase-free water	Variable
Template DNA (added at step 4)	Start with 10 ng (use 30 pg–30 ng as the range)
Total reaction volume	**25**§

‡ Delivered at a concentration of 20x.

§ If the real-time cycler requires a final reaction volume other than 25 µl, adjust the amount of master mix and all other reaction components accordingly. If using 384-well plates on the ABI PRISM 7900, use a reaction volume of 10 µl.

Table 3. Reaction setup for triplex and 4-plex PCR

Component	Volume (µl)
Reaction mix 2x Type-it Probe PCR Master Mix	12.5
25x Type-it CNV Reference Probe Assay solution	1
25x primer–probe mix for GOI 1*	1
25x primer–probe mix for GOI 2*	1
Only for 4-plex PCR: 25x primer–probe mix for GOI 3*	1
RNase-free water	Variable
Template DNA (added at step 4)	Start with 10 ng (use 30 pg–30 ng as the range)
Total reaction volume	**25**†

* **IMPORANT**: For triplex and 4-plex RT-PCR, a 25x primer–probe mix consists of 10 µM forward primer, 10 µM reverse primer, and 5 µM probe in Buffer TE, resulting in a final concentration of 0.4 µM forward and reverse primer, and 0.2 µM probe.

† If the real-time cycler requires a final reaction volume other than 25 µl, adjust the amount of master mix and all other reaction components accordingly. If using 384-well plates on the ABI PRISM 7900, use a reaction volume of 10 µl.

Table 4. Cycling conditions

Step	Time	Temperature
PCR initial activation step	5 min	95°C
2-step cycling: Denaturation	30 s	95°C
Annealing/extension	30 s‡	60°C
Number of cycles	40	

‡ For real-time PCR systems that require a minimum annealing/extension time longer than 30 seconds, adjust the time accordingly, as per the requirements of the real-time PCR system in operation.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.