Type-it® CNV SYBR® Green PCR+ qC Kit and Type-it CNV SYBR Green PCR Core Kit

The Type-it CNV SYBR Green PCR+ qC Kit (cat nos. 206672 and 206674) and the Type-it CNV SYBR Green PCR Core Kit (cat. no. 206624), 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 SYBR Green 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 SYBR Green PCR +qC 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 Type-it CNV SYBR Green Kits 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, SYBR Green-based 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 Primer Assay as a universal reference assay for reliable $\Delta\Delta C_T$-based quantification of the CNV in the human genome. Note that the Type-it CNV Reference Primer Assay is not provided with the Type-it CNV SYBR Green PCR Core Kit.
- We recommend preparing a 25x primer mix containing specific primers (recommended concentrations in the 25x primer mix: 25 µM each primer) for each GOI. See Table 1 for details.
- To reconstitute the Type-it CNV Reference Primer 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.

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- Always use 30 pg–30 ng template DNA (see Table 1) and the optimized cycling conditions specified in Table 2.
- The PCR must start with an initial incubation step of 5 min at 95°C to activate HotStarTaq® *Plus* DNA Polymerase

1. Thaw the 2x Type-it SYBR Green PCR Master Mix, template DNA, Type-it CNV Reference Primer Assay 25x working solution, 25x primer mix containing primers for the gene of interest (GOI), and RNase-free water. Mix the individual solutions and place them on ice.
2. Prepare a reaction mixture according to Table 1.
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 Table 1 for details.
5. Program the real-time cycler according to Table 2.
6. Place the PCR tubes, Rotor-Disc, or plate in the real-time cycler, and start the cycling program.
7. Perform data analysis.

   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

Component	Volume (µl)
Reaction mix 2x Type-it SYBR Green PCR Master Mix	12.5
25x Type-it CNV Reference Primer Assay or 25x primer mix for GOI	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**: 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. Cycling conditions

Step	Time	Temperature
PCR initial activation step	5 min	95°C
2-step cycling: Denaturation	10 s	95°C
Annealing/extension	30 s†	60°C
Number of cycles	35	

† 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.

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