PrimePCR™ ddPCR™ Copy Number Variation (CNV) Assay

For research use only. Not for diagnostic purposes.

Ordering Information
PrimePCR™ assays for ddPCR can be ordered only online at www.bio-rad.com/primepcr

Storage and Stability
The ddPCR assays are stable for 12 months when stored at 4°C protected from light. The 20x assay mix can be kept at –20°C for long-term storage.

Kit Contents
The PrimePCR for ddPCR CNV assay is a 20x concentrated, ready-to-use primer-probe mix optimized for use with droplet PCR supermix. Each kit comes with 200 µl, 1,000 µl, or 2,500 µl of the 20x assay mix (18 µM primers and 5 µM probe), sufficient for 200, 1,000, or 2,500 x 20 µl reactions, respectively.

Other Required Material & Instruments
20x PrimePCR ddPCR reference assays [For CNV, target assays are run duplexed to a reference assay (HEX)]
QX100™ droplet generator (186-3002)
QX100™ droplet reader (186-3003)
C1000 Touch™ thermal cycler (185-1196)
PX1™ PCR plate sealer (181-4000)
Please refer to instrument-specific manuals for ordering information for other consumables (oils, cartridges, gaskets, plates and seals).

Protocol
Step 1: Restriction Digest of Sample DNA

- DNA fragmentation by restriction digestion is necessary for optimal accuracy in copy number analysis by ddPCR. Restriction digest separates tandem gene copies, reduces sample viscosity, and can improve assay performance by improving template accessibility. PrimePCR ddPCR CNV assays are designed specifically to work universally with HaeIII restriction digest
- An example restriction digest using HaeIII is shown in Table 1. Digest using HaeIII at 37°C for 1 hour. Do not heat inactivate following digest. DNA purification is not necessary after restriction digestion. Use a minimum tenfold dilution of the digest to reduce the salt content of the sample in ddPCR. Store digested DNA at –20°C

Table 1. Example HaeIII restriction enzyme digestion

Reaction Component	Volume per Reaction	Final Concentration
10x CutSmart Buffer*	5 µl	1x
Genomic DNA (1-5 ug)	Variable	20–100 ng/µl
Restriction endonuclease	Variable	10 U/µg genomic DNA
Water	Variable	—
Total	**50 µl**	—

*NEB recently changed their restriction digest buffer formulations. HaeIII is compatible with NEB4 buffer or the new CutSmart Buffer. This enzyme does not require addition of BSA.

Step 2: ddPCR Reaction Setup

- Thaw all frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solutions at the bottom of tubes, and then store on ice protected from light
- Prepare samples at room temperature according to recommendations in Table 2 If multiple samples are to be assayed using the same target and reference duplex, prepare a master reaction mix without sample template, dispense equal aliquots into the reaction tubes, and add the sample template to each reaction tube as the final step. Mix by pipetting up and down 5-10 times to ensure full addition of sample DNA
- For most routine CNV applications, where a diploid target copy number is expected to be 10 or less, approximately 10 – 66 ng of human genomic DNA should be added per ddPCR well

Table 2. ddPCR Reaction Setup		
Component	**Volume per Reaction**	**Final Concentration**
2x droplet PCR supermix	10 µl	1x
20x target primer/probe mix (FAM)	1 µl	900 nM primers/250 nM probe
20x reference primer/probe mix (HEX)	1 µl	900 nM primers/250 nM probe
HaeIII-digested DNA template (10 – 66 ng)	Variable	Variable
RNase/DNase-free water	Variable	--
Total volume	**20 µl**	**--**

- Vortex reaction mixture thoroughly, spin down, and dispense 20 µl of the mix into the sample well of the QX100 droplet generator cartridge. Follow general guidelines for droplet generation
- After droplet generation using a QX100 droplet generator, transfer reaction mix onto the recommended 96-well PCR plate.
- Program thermal cycling protocol on the C1000 Touch thermal cycler according to Table 3.
- Load the PCR plate onto the thermal cycler and start the PCR run. After thermal cycling, transfer PCR reaction plate onto a QX100 droplet reader and follow instrument-specific guidelines

Table 3. Thermal Cycling Protocol*				
Cycling Step	**Temperature**	**Time**	**Ramp Rate**	**# Cycles**
Enzyme activation	95°C	10 min	~2°C/sec	1
Denaturation	94°C	30 sec		40
Annealing/extension	60°C	1 min		
Enzyme deactivation	98°C	10 min		1
Hold (optional)	4°C	infinite	~1°C/sec	1

*Use a heated lid set to 105°C and set the sample volume to 40 µl.

Other Recommendations

- If 10-50 copies per diploid genome are expected in a sample, add 15ng or less of sample per well. For copy number evaluation above 50 copies per diploid genome, strategies using multiple wells can be used
- When running technical replicate wells, assemble a common reaction mix (enough for 1.5x as many wells as you plan to run) with all required components and sample template
- The ddPCR supermix for probes can be used with PrimePCR ddPCR CNV assays, is compatible with uracil N-glycosylase (UNG) for preventing carryover contamination, and may be purchased from a licensed supplier

Quality Control

The ddPCR CNV assay is free of detectable DNase and RNase activities. The assay demonstrates greater than 90% precision and the ability to effectively differentiate between PCR-positive and PCR-negative droplets. Stringent specifications are maintained to ensure lot-to-lot consistency.

To learn more about Bio-Rad's complete solution for amplification, visit our website: **www.bio-rad.com/amplification.**

1 0 0 4 6 5 0

Bio-Rad Laboratories
2000 Alfred Nobel Drive, Hercules, CA 94547
510-741-1000