BIO-RAD

ddPCR™ *SMN2* Copy Number Determination Kit

Catalog #	Description
186-3503	**ddPCR *SMN2* Copy Number Determination Kit**, 200 x 20 µl reactions

For research purposes only.

Description

The ddPCR *SMN2* Copy Number Determination Kit is designed to determine the copy number of the *SMN2* gene only, one of two highly similar genes that play a pivotal role in spinal muscular atrophy — an autosomal recessive neuromuscular disorder.

Storage and Stability

The Droplet Digital™ PCR (ddPCR) *SMN2* Copy Number Determination Kit is stable through the expiration date printed on the label when stored at –20°C and protected from light. Repeated freezing and thawing of the kit is not recommended.

Kit Contents

Each kit includes 200 µl of 20x ddPCR *SMN2* Copy Number Determination Assay, three positive controls (2, 3, and 4 copy *SMN2* positive controls), and two tubes of 2x ddPCR Supermix for Probes (No dUTP), sufficient for 200 reactions. The 20x ddPCR *SMN2* Assay includes reagents to detect both the *SMN2* gene (in FAM) and a reference gene (in HEX). The reference assay targets the *RPP30* gene.

Required Equipment

- QX100™ or QX200™ Droplet Generator (catalog #186-3002 or 186-4002, respectively) or Automated Droplet Generator (catalog #186-4101)
- QX100 or QX200 Droplet Reader (catalog #186-3003 or 186-4003, respectively)
- C1000 Touch™ Thermal Cycler with 96–Deep Well Reaction Module (catalog #185-1197)
- PX1™ PCR Plate Sealer (catalog #181-4000)

Please refer to the QX100 or QX200 Instruction Manuals (#10026321 and 10026322 or 10031906 and 10031907, respectively) or the Automated Droplet Generator Instruction Manual (#10043138) for ordering information on consumables (oils, cartridges, gaskets, plates, and seals).

Protocol

1. Thaw frozen reaction components to room temperature. Mix thoroughly by vortexing, and then centrifuge to remove any concentration gradient that may have formed during –20°C storage.
2. Prepare a master ddPCR Reaction Mix for the appropriate number of controls and samples to be screened according to the guidelines in Table 1.

Table 1. Preparation of the master ddPCR Reaction Mix.

Component	Volume for 8 Wells, µl	Volume for 48 Wells, µl	Volume for 96 Wells, µl
2x ddPCR Supermix for Probes (No dUTP)	92	552	1,104
20x ddPCR *SMN2* Assay	9	54	108
HaeIII (10 U/µl)	2.5	15	30
Nuclease-free water	49	294	588
Final volume (µl)	**152.5**	**915**	**1,830**

3. Thoroughly mix the master ddPCR Reaction Mix.
4. Pipet 18 µl of the master ddPCR Reaction Mix into the required number of wells of the 96-well plate.
5. Following the plate layout in Table 2, pipet 4 µl of positive control DNA, purified genomic DNA (gDNA), or sample extraction/storage buffer for the positive controls, samples, and no template control (NTC) wells, respectively.
6. For the most accurate copy number measurements, load gDNA samples that are 6–35 ng/µl.
7. For any wells within a column not containing controls or samples, add 20 µl 1x ddPCR Buffer Control (catalog #186-3052).
8. Thoroughly mix the control DNA, samples, and NTC buffer into the master ddPCR Reaction Mix.

Table 2. Plate layout for *SMN2* copy number determination.

	1	2	3	4	5	6	7	8	9	10	11	12
A	2 Copy *SMN2* Control	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37	Sample 45	Sample 53	Sample 61	Sample 69	Sample 77	Sample 85
B	3 Copy *SMN2* Control	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38	Sample 46	Sample 54	Sample 62	Sample 70	Sample 78	Sample 86
C	4 Copy *SMN2* Control	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	Sample 47	Sample 55	Sample 63	Sample 71	Sample 79	Sample 87
D	No Template Control	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	Sample 48	Sample 56	Sample 64	Sample 72	Sample 80	Sample 88
E	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41	Sample 49	Sample 57	Sample 65	Sample 73	Sample 81	Sample 89
F	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34	Sample 42	Sample 50	Sample 58	Sample 66	Sample 74	Sample 82	Sample 90
G	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35	Sample 43	Sample 51	Sample 59	Sample 67	Sample 75	Sample 83	Sample 91
H	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36	Sample 44	Sample 52	Sample 60	Sample 68	Sample 76	Sample 84	Sample 92

9. Load 20 µl of each reaction mixture into a sample well of a DG8™ Cartridge for QX200/QX100 Droplet Generator (catalog #186-4008) followed by 70 µl of Droplet Generation Oil for Probes (catalog #186-3005) into the oil wells, according to the QX100 or QX200 Droplet Generator Instruction Manual (#10026322 or 10031907, respectively). For the Automated Droplet Generator, follow instructions in the Automated Droplet Generator Instruction Manual (#10043138).
10. Carefully transfer the generated droplets into a clean 96-well plate. For the Automated Droplet Generator, remove the droplet plate containing ddPCR droplets. Seal the plate with the recommended foil seal and a PX1 PCR Plate Sealer.
11. Perform thermal cycling of droplets using a C1000 Touch Thermal Cycler with 96–Deep Well Reaction Module according to the protocol shown in Table 3.

Table 3. Thermal cycling protocol.*

Cycling Step	Temperature, °C	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	10 min	2°C/sec	1
Denaturation	94	30 sec		40
Annealing/extension	55	1 min		40
Enzyme deactivation	98	10 min		1
Hold (optional)	12	Infinite		1

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

Data Analysis

1. After thermal cycling, place the sealed ddPCR Plate into a QX100 or QX200 Droplet Reader. Refer to the QX100 or QX200 Droplet Reader and QuantaSoft Software Instruction Manual (#10026321 or 10031906, respectively).
2. Open QuantaSoft™ Software and set up the plate template according to the plate map in Table 2. In the well editor, select **CNV2** (for two reference copies per genome) as the experiment type. Label Target 1 *SMN2* and select **Ch1 Unknown** as the type. Label Target 2 *RPP30* and select **Ch2 Reference** as the type. For QuantaSoft Software version 1.4 or later, select **ddPCR Supermix for Probes (No dUTP)** as the supermix type.
3. Click **Run** and select **FAM/HEX** as the dye set.
4. After data acquisition, in the Analyze tab, select all the wells. Examine any wells with a CHECK status in the data table. Those with an error other than Automatic Analysis Unsuccessful should be omitted from further analysis.
5. Click the **2-D Amplitude** tab and set a universal threshold for the selected wells by manually applying a multiwell threshold between the positive and negative droplets as shown in the amplitude plot of Figure 1. Refer to the QX100 or QX200 Droplet Reader and QuantaSoft Software Instruction Manual (#10026321 or 10031906, respectively) for more information on thresholding.

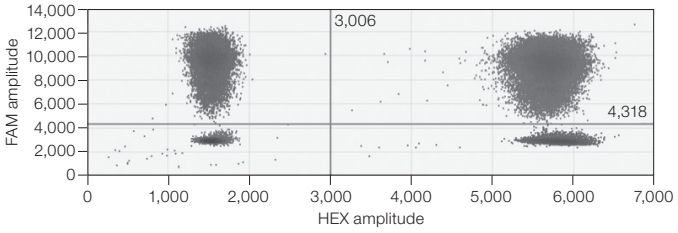

Fig. 1. Universal threshold set across all wells.

6. Use the data table and select only those samples that have 100–5,000 copies per microliter in both channels. Samples outside this range should be repeated at a more appropriate concentration.
7. Click the **Concentration** tab and confirm the No Template Control well (D1) has <0.5 copies/µl for both *SMN2* and *RPP30*. If this well contains >0.5 copies/µl of either *SMN2* or *RPP30*, care should be taken in interpreting the experimental results because this suggests potential contamination (see Troubleshooting section).
8. Click the **Copy Number** tab and confirm wells A1 (2 Copy *SMN2* Control), B1 (3 Copy *SMN2* Control), and C1 (4 Copy *SMN2* Control) have two, three, and four copies of *SMN2* (±10%) respectively. If any of these controls are outside the expected range, care should be taken in interpreting the experimental results because this suggests an error in experimental setup, execution, or analysis.

Troubleshooting

1. If >0.5 copies/µl are positive for *SMN2* in well D1, please follow standard laboratory practices to minimize PCR contamination.
2. If the measured *SMN2* copy number in wells A1, B1, and C1 are outside the allowable 10% error from the integer value (2, 3, and 4, respectively), then use a new batch of HaeIII restriction enzyme and ensure the reaction mixtures are thoroughly mixed prior to droplet generation.
3. If a clean threshold cannot be uniformly set for the controls (wells A1–D1), check the expiration date on the kit, evaluate whether the kit has been properly stored, and confirm the thermal cycling conditions used.
4. If a clean threshold cannot be set on the samples but can be set for the controls, consider using a different DNA extraction method to remove inhibitors that may be present in the samples. Also, confirm the sample is gDNA purified from fresh blood or tissue and is not DNA isolated from plasma or a formalin-fixed, paraffin-embedded sample, which may be highly degraded and result in poor distinction between negative and positive droplets.
5. If concentration and copy number variation estimates between replicates are variable, ensure thorough mixing of the samples into the master ddPCR Reaction Mix.

Quality Control

ddPCR *SMN2* Copy Number Determination Kits are free of detectable DNase and RNase activity. Stringent specifications are maintained to ensure lot-to-lot consistency.

Visit **bio-rad.com/ddPCRSMN2** for more information.

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