

SMITEST

Parvovirus B19 DNA Detection Kit Ver.2

- ◆ Can be used for detecting **all parvovirus genotype 1, 2, and 3** in serum and plasma samples
- ◆ High sensitivity and specificity: minimum detection sensitivity- **5 IU/test**
- ◆ Optimized for processing many samples: **simultaneous amplification for 96 samples**

Assay principle

The SMITEST Parvovirus B19 DNA detection kit is intended for the detection of the Parvovirus B19 DNA in a sample by PCR, amplification, hybridization, and enzyme immune assay method. Parvovirus B19 specific primers are used for gene amplification and the amplified products are detected in a micro cup coated with Parvovirus B19 specific probes. The internal control DNA is co-amplified with Parvovirus B19 specific primers at the same time in a vial, and the amplified control DNA is hybridized in the micro cup coated with internal control probes then confirms the amplification performance.

Assay procedure

1) Sample preparation

Serum or plasma :100 μ L
Extraction with SMITEST EX R&D (recommendation)



Dried DNA pellet after extraction

Specimen diluent

25 μ L of the specimen diluent



Dissolve the DNA pellet with 25 μ L of the specimen diluent.

2) PCR amplification



Extracted DNA, Master Mix, Internal control, Taq DNA polymerase*, Uracil DNA Glycosylase*
*not supplied with the kit and ask for recommended reagents

25 μ L of the specimen diluent + 25 μ L of DNA sample (Total 50 μ L / test)

PCR amplification

40°C 5 min.
94°C 1 min.
94°C 20 sec. ▶ 63°C 20 sec. ▶ 72°C 10 sec. 20 cycles
90°C 20 sec. ▶ 60°C 20 sec. ▶ 72°C 10 sec. 50 cycles
94°C 10 min.
72°C forever

DNP labeled primer
Parvovirus B19 DNA

DNP labeled primer
Internal control plasmid



Extracted parvovirus B19 DNA and internal control are amplified with specifically designed DNP-labeled primers.

3) Hybridization

Add immediately 50 μ L of the Denaturation Solution to PCR product.

Incubate for 10 min. at room temperature.

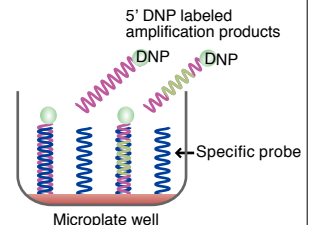


Dispense 25 μ L of denatured PCR product to the microplate, in which 100 μ L of the Hybridization Buffer is already dispensed in each well.

Incubate for 10 min. at 37°C.

Wash 5 times with 350 μ L of the wash solution per well.

Denaturation Solution, Probe coated microplate, Hybridization buffer, 20x Wash Concentrate



The probes designed against sequence of virus DNA and internal control plasmid are coated on a microplate well. During incubation, 5' DNP labeled amplification products are hybridized with the probes on the well.

4) Detection by EIA

Anti-DNP antibody-HRP conjugate 100 μ L
Incubate at 37°C 30 min.

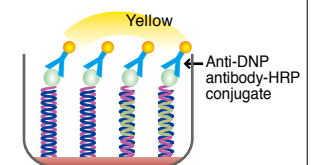
Wash the wells 5 times with 350 μ L of the wash solution.

Substrate 100 μ L
Incubation at room temperature, dark place for 15 min.

Stop reagent 100 μ L

Read absorbance (450 nm).

Anti-DNP antibody-HRP conjugate, Substrate, Stop reagent, 20x Wash Concentrate



The DNP-label amplified products hybridized with HRP conjugated anti-DNP antibodies are detected colorimetrically by HRP-TMB reaction.

Kit performance

Cut off value : 0.2

Minimum detection sensitivity : 5 IU/test

<Interpretation>

Two wells per one sample are required.

P: Parvovirus B19 test well

IC: Internal control well

Yellow is developed when sample is positive.

When it is negative, no color is developed.

* IC well is used for confirmation of optimal amplification performance.

In the case of only IC well being positive, interpretation of sample is implemented.

In the case of IC well does not develop color, failure in the amplification must be taken into consideration, and a re-test must be performed.

	Genotype : 1		2		3		
	P	IC	P	IC	P	IC	
5 IU/test	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	← Positive P/IC : Yellow
0 IU/test	No color	Yellow	No color	Yellow	No color	Yellow	← Negative P : No color IC : Yellow

<Detection sensitivity>

Genotype 1 close to 5 IU/test 6-time positives in 6-time assays

Genotype 2 close to 5 IU/test 6-time positives in 6-time assays

Genotype 3 close to 5 IU/test 6-time positives in 6-time assays

Negative 0 IU/test all negatives in 6-time assays

	Genotype 1 (5 IU)		Genotype 2 (5 IU)		Genotype 3 (5 IU)		Negative (0 IU)	
	P	IC	P	IC	P	IC	P	IC
1	>3	>3	>3	>3	>3	>3	0.033	>3
2	>3	>3	>3	>3	>3	>3	0.029	>3
3	>3	>3	>3	>3	>3	>3	0.023	>3
4	>3	>3	>3	>3	>3	>3	0.027	>3
5	>3	>3	>3	>3	>3	>3	0.021	>3
6	>3	>3	>3	>3	>3	>3	0.040	>3

We made positive control plasmids and used them in this assay.

We confirmed that the positive control plasmids have the same performance in the sensitivity as NIBSC 99/800 and 99/802.

Kit components

	Components	Quantity
Store at 2-8°C	Denaturation solution	1x 6 mL
	Hybridization buffer	1 x 24 mL
	Anti-DNP antibody HRP conjugate	1 x 24 mL
	Substrate	1 x 24 mL
	Stop reagent	1 x 24 mL
	20x Wash concentrate	1 x 100 mL
	Parvovirus B19 probe coated microwell plate	1 x plate
	Internal control probe coated microwell plate	1 x plate
Store at -20°C	Specimen diluent	3 x 1.7 mL
	Master mix	2 x 1.25 mL
	Internal control solution	1 x 110 µL



* Reagents required but not supplied –Recommended items–

- AmpliTaq DNA polymerase (5 units/µL): Applied Biosystems, part No. N8080160 or N8080171
- Uracil DNA Glycosylase (UDG) (1 U/µL): Invitrogen, cat. No.18054015

CodeNo.	Product	Quantity	Storage
GS-B0521	SMITEST Parvovirus B19 DNA Detection Kit Ver.2	96 tests	Store at 2-8°C
GS-B0522		96 tests	Store at -20°C