VDx® BLV PCR
VDx® BLV nested PCR
Cat. No. NB-BLV-11, NB-BLV-12

1. Description

VDx® BLV PCR / Nested PCR Kit is provides a range of testing for the detection of Bovine Leukemia Virus(BLV) by PCR method.
VDx® PCR Kit is formulated for the maximal stability of Taq polymerase(and Reverse Transcriptase) that has been dried together with reaction buffer, dNTP and stabilizer. It contains loading dyes for further convenience of use. Thus, the reaction mixtures after PCR cycles are ready for agarose gel electrophoresis. VDx® PCR Kit is ready-to-use PCR mixture containing concentration of components required for PCR in one tube. For reactions, simply add template (DNA) and primer mix.

2. Storage

The components of VDx® BLV PCR / Nested PCR Kit should be stored at -20°C, under this condition, the kit is stable until expiration date stated on the label.

3. Contents

Reagents	BLV PCR	Nested PCR
PCR Premix	96T	96T
Control DNA	100㎕ X 1	-
BLV Primer mix	800㎕ X 2	-
BLV nested Primer mix	-	800㎕ X 2
Instruction manual	1ea	1ea

4. Template preparation

4.1 Target Sample : white blood cells, whole blood and lesion tissue(The samples should be kept as fresh as possible and frozen during storage.).
4.2 Template genes are extracted from 100~300㎕ of sample using QIAmp Viral RNA Mini Kit (Qiagen). Refer to the manufacturer's instructions for gene extraction methods.
*The gene extraction kit can be used with other products, but please check the manufacturer's manual in advance.

5. PCR method

❖ BLV PCR (#NB-BLV-11)

1) Prepare appropriate PCR Premix tubes.
2) Add 15㎕ of primer mix solution into PCR premix tube.
3) Add 5㎕ of template DNA into the PCR premix tube.
4) Gently mixed and briefly centrifuged.
5) Perform PCR reaction of samples as the below process using PCR machine.

❖ BLV Nested PCR (#NB-BLV-12)

1) Prepare appropriate PCR Premix tubes.
2) Add 19㎕ of primer mix solution into PCR premix tube.
3) Add 1㎕ of template DNA(PCR products by BLV PCR) into the PCR premix tube.
4) Gently mixed and briefly centrifuged .
5) Perform PCR reaction of samples as the below process using PCR machine.

6. Detection of Amplified Products

1) Prepare 1.5% agarose gel containing Ethidium Bromide (Et-Br).
2) Load 5 ㎕ of PCR product on agarose gel without adding a loading dye buffer and perform electrophoresis.
3) Run electrophoresis by 100V(required about 20~40min).
4) Identify the result on ultra-violet(UV) transilluminator.

Step	BLV PCR (#NB-BLV-11)			Nested PCR (#NB-BLV-12)		
	Temp	Time	Cycle	Temp	Time	Cycle
Initial inactivation	94℃	3 min	1 cycle	94℃	3 min	1 cycle
Denaturation	94℃	20 sec	40 cycles	94℃	20 sec	25 Cycles
Annealing	55℃	20 Sec		55℃	20 sec	
Extension	72℃	30 sec		72℃	30 sec	
Final Extension	72℃	5 min	1 cycle	72℃	5 min	1 cycle

❖ Caution : Use of Control DNA

1) The control DNA contained in the product is designed to amplify the 679bp gene.
2) If a 679bp gene is detected in the sample, it can be judged that the Control DNA is contaminated.
3) Control DNA is used as a control reagent to check whether gene amplification occurs. It is added last using Filter tip to prevent contamination with sample.

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7. Interpretation

1) Control DNA can identify amplified products of 679bp.
2) If the band of the below size is confirmed in the sample below, it is judged as the POS.

Product	Virus	Target gene	Size
BLV PCR	BLV	env	551 bp
	Control DNA		679 bp
BLV Nested PCR	BLV	env	367 bp

8. Notice

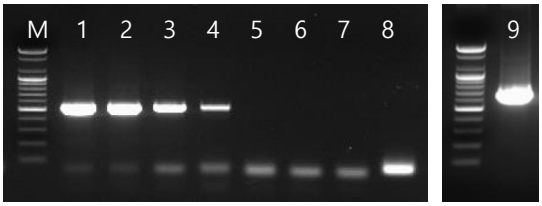
- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only.*
- Do not use any reagent after the expiration date.
- Do not use it with reagents of other products.

9. Trouble shooting

1) In the case of difficult to interpret results due to non-specific bands
   -> Reduce amount of template by 1/10 dilution and reacts again.
2) Preparation of PCR reaction at room temperature may cause the non-specific band.
3) All procedure should be carried out on ice.

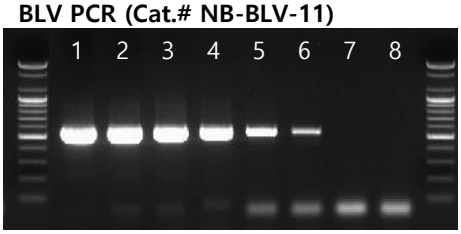
Gel Electrophoresis (1.5% Agarose)

BLV PCR (Cat.# NB-BLV-11)



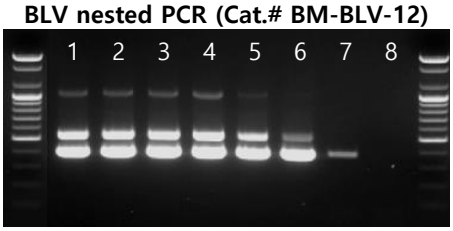
M: Size Marker
Lane 1 : BLV (551bp)
Lane 2 : BLV (551bp)
Lane 3 : BLV (551bp)
Lane 4 : BLV (551bp)
Lane 5~8 : Negative sample
Lane 9 : Control DNA

BLV PCR (Cat.# NB-BLV-11)



Nested PCR

BLV nested PCR (Cat.# BM-BLV-12)



M: Size Marker
Lane 1~7 : BLV positive sample
Lane 8 : Negative sample

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