REF:PCR-V344-48D PCR-V344-96D

# VetPCR<sup>™</sup> Minute virus or mice Detection

#### **SYMBOLOGY**



REF Catalog Number

Limits Temperature **Expiration Date** 

Consult Instructions for Use

# 1. DESCRIPTION

The autonomous parvovirus minute virus of mice (MVM) contains a linear, single-stranded DNA genome. Minute virus of mice (MVM) is one of the most frequent viral pathogens in laboratory mice. The virus can be shed from infected mice via feces and urine, but also via fomites and nasal secretions. Typically there are no clinical signs of infection, however, experimental infection can cause multiple organ damage during fetal development or shortly after birth. While MVM infection may result in pathology of infected mice, the infection is more likely to cause problems for the ongoing study the mice are being used for as the immune system will be activated, the activity of T-lymphocytes and B-lymphocytes will be altered and tumor formation may be suppressed.

VetPCR Minute virus or mice detection Kit is the direct detection of Minute virus or mice on the basis of a genetic database, so it can diagnose very fast and accurately. It can amplify only specific gene using the PCR (Polymerase Chain Reaction) method, and take only 3 hours for detection. Therefore, it is a very fast, accurate, and reliable technique.

# 2. STORAGE

The VetPCR<sup>™</sup> Minute virus or mice Detection Kit is shipped at room temperature (15-25°C) because contains a chemical stabilizer.

The VetPCR™ Minute virus or mice Detection Kit should be stored immediately upon receipt at -20°C in a constant-temperature freezer. For routine use should be stored al 4°C. When stored under these conditions and handled correctly, these products can be kept at least until the expiration date without showing any reduction in performance.

# **3. KIT CONTENTS**

KIT	48	96	
VetPCR <sup>™</sup> Minute virus or mice Premixture		1	vial
PCR Internal Control (white cap)		1	vial
DNase/RNase free water	1	1	vial
Minute virus or mice PCR Positive control	1	1	vial
PCR Negative control	1	1	vial
Mineral Oil Solution	1	1	vial
Brig™ Molecular Weight marker	1	1	vial
DNA purification kit	50	100	test

#### **4.MATERIALS**

Materials required but not provided:

- Microcentrifuge and PCR tubes
- Disposable gloves, powderless
- Pipettes
- · Sterile pipette tip

- Vortex mixer
- · Centrifuge for microcentrifuge tubes
- Thermal cycler
- Tube racks
- UV transilluminator · Biohazard waste container

#### 5.PROCEDURE

Please read through the entire procedure before starting. **5.1 DNA PREPARATION** 

This kit includes all reagents necessary for purification of DNA from different samples. Carry out the DNA isolation according to the instructions available inside the kit, the instructions also can be downloaded of our website www.bioingentech.com. If you need an additional DNA Purification kit, use only the following kits that are standardized for the process: See Item 9. ADDITIONAL PRO-DUCTS.

# Note: /!\

Completely thaw the components of the kit prior to use; homogenize the solutions for several seconds prior to pipetting.

# 5.2 PREPARATION OF Minute virus or mice PCR MIXTURE

1) Prepare the reaction mixture for sample, positive control, negative control, and internal control by combining the reagents as shown in the table 1. The final reaction volume should be 13.5µl.

Notes:

#### · Run a positive control, a negative control, and an internal control each 12 samples.

• The mineral oil is necessary, even when using a thermal cycler that employs a top heating method.

#### Table 1. Reaction components for PCR

Kit components	Sample	Positive control	Negative control	Internal control
VetPCR™ Minute virus or mice Premixture	5.5µ L	5.5µL	5.5µL	
PCR Internal control (white cap)	]			5.5µL
DNase/RNase free water	6µ1	6µI	6µI	6µ1
DNA isolated from the sample	2µ1			2µ1
Minute virus or mice Positive control		2µI		
PCR Negative control			2µI	
Mineral Oil Solution	11µI	11µ1	11µ1	11µI

2) Place the tubes in a thermal cycler and perform amplification according to the program outlined in Table 2.

Table 2. PCR cycling parameters

PCR cycle		Temp.	Time
1 cycle	Initial Denaturation	94°C	2 min.
	Denaturation	94°C	30 sec.
30 cycles	Annealing	58°C	30 sec.
	Extension	72ºC	30 sec.
1 cycle	Final extension	72ºC	5 min.

Eloingentech Ltd. • Bernardo O'Higgins 1186, Of. 1307 • Concepción, Chile • Phone/Fax (56)-(41)-2790435 • www.bioingentech.com • info@bioingentech.com

#### 5.3. DETECTION OF AMPLIFIED PRODUCTS

Prepare 1.5% agarose gel containing Ethidium bromide (Et-Br).
 Load 7µI of PCR product, 7µI of positive control, 7µI of negative control, 7µI of internal control and 2µI of Brig<sup>™</sup> Molecular Weight marker on agarose gel without adding a loading-dye buffer and perform electrophoresis.

3) Run electrophoresis by 100V (required about 30~40 minutes).

4) Identify the result on ultra-violet (UV) transilluminator.

#### 5.4. INTERPRETATION OF THE TEST RESULTS

- Expected PCR product size: 261bp

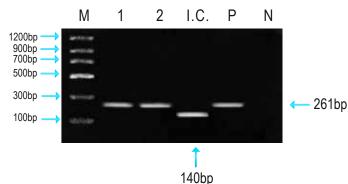


Fig. 1 Result:

Lane M: Brig<sup>™</sup> Molecular Weight Marker (Bioingentech Ltd.) Lane 1~2: Minute virus or mice Positive samples Lane I.C.: Internal control Lane P: Positive control Lane N: Negative control

#### 6. TROUBLESHOOTING

#### 1) No band in positive sample

• Check Internal control band: If internal control band is seen, PCR has been performed properly. It is not a problem of the product.

• Check template DNA quality: the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with DNA rehydration solution and used to perform PCR again.

• Check PCR machine: check the temperature and make sure to check that the machine is working properly.

#### 2) No internal control band

Check template DNA concentration: Competition can occur by high template concentration. Proceed with a lower concentration of DNA.
Check template DNA quality: Even tough DNA is isolated from the sample, the PCR reaction can be inhibited depending on DNA purity in some cases. In this case, extracted DNA should be diluted 10 times with distilled water and used to run the PCR reaction again. If still no band is seen, please inquire with our technical support staff.

#### 3) Amplicon bands in the negative control

• Check contamination of distilled water: Distilled water can be contaminated. Perform PCR again with fresh sterile water.

• Check contamination of laboratory instruments and other environments: We recommend that you use filter tips and a pipette after sterilization to reduce contamination. Proceed with all procedures on a clean bench and keep the location where you procedures are performed sterile.

#### 4) Poor resolution on agarose gel

• We recommend using a 1.5~2% agarose gel and run electrophoresis for 40 minutes at 100 V.

#### 7. TECHNICAL ASSISTANCE

At Bioingentech we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of Bioingentech products. If you have any questions or experience any difficulties regarding Bioingentech Genomic DNA Detection Kits or Bioingentech products in general, please do not hesitate to contact us.

#### 8. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS), available online in our website, where you can find, view, and print the MSDS for each Bioingentech kit and kit component.

#### 9. ADDITIONAL PRODUCTS

Product	Product Code	Manufacturer
Bioingentech™ Genomic DNA Purification Kit (50 test)	PU-A001	Bioingentech
Bioingentech™ Genomic DNA Purification Kit (100 test)	PU-A002	Bioingentech

Product use limitations warranty disclaimer

2004 – 2018 Bioingentech corporation. All rights reserved.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Bioingentech technical services or access the Bioingentech online catalog for the most up-to-date information on Bioingentech products.