BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Fowl adenovirus type 11 (FADV11) detection kit (qPCR, Exogenous IPC)

Catalog number: VD1430-2

This package insert must be read in its entirety before using this product.
For Veterinary Use Only.

BOSTER
antibody and ELISA experts

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Fowl adenovirus type 11 (FADV11) detection kit (qPCR, Exogenous IPC)

Catalog Number: VD1430-2

Product Overview

Fowl adenovirus type 11 (FADV11) is a DNA virus, infectious to various birds. VD1430-2 is an easy to use, highly sensitive qPCR assay that detects FADV11. This kit can detect up to 48 individual samples or pooled samples. This kit is sensitive and specific for detecting FADV11 and can be used for reliable screening and monitoring of FADV11 outbreaks.

Assay Principle

The kit contains fluorescent primers and probes designed to specifically target FADV11. The primers and probes amplify the FADV11 in the sample and the amount of fluorescence during PCR cycles is measured to determine the amount of FADV11.
Primers and probes against exogenous internal positive control (IPC) are included. The controls should always generate positive results. Negative results from IPC indicate issues with either sample preparation or PCR amplification, usually due to presence of PCR inhibiting substances in the samples.

Kit Features

High specificity: This kit utilizes our proprietary technology where 3 monoclonal antibodies are used to block and dampen the activity of the Taq polymerases to prevent non-specific replication of DNA in lower temperatures. This makes the kit compatible with a wider range of sample types.
High sensitivity: This kit contains dNTPs, PCR enhancers and stabilizers, all optimized for maximize the sensitivity of PRV detection.
Minimal cross-contamination: This kit incorporates the dUTP/UDG system, which degrades the ssDNA and dsDNA that contains Uracil (U), eliminating PCR product-induced cross-contamination.

Kit Components

#	Component	Ingredients	Volume
1	Ready to use PreMix	qPCR reaction buffer, dNTPs, UDG enzymes, Taq polymerases, primers, probes	960 uL/tube
2	Negative Control	Enzyme-free water	400 uL/tube
3	IPC	IPC fragments	480 uL/tube
4	Positive control	Target gene fragments	50 uL/tube

Compatible Sample Types

Tissue samples such as livers, spleens, and kidneys suspected to be infected with dead poultry.

Storage Conditions

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

Ship in dry ice. Store at -20°C for 12 months, store in the dark, avoid repeated freezing and thawing.

* For Veterinary Use Only.

Protocol

Sample collection and preparation

Follow lab best practices for PCR (details see end of this document) in collecting and handling samples. Samples should be processed using commercially available Nucleic acid extraction kits to isolate the total Nucleic acid in the samples. Before nucleic acid extraction, for each 200 uL negative control, and for each 200 uL sample, add 10 uL IPC gene fragments.

The extracted nucleic acid must be used fresh or stored in -20 °C for later use, and not exceeding 6 months.

If before nucleic acid extraction IPC gene fragments were not added, alternatively IPC can be added to the extracted nucleic acid. This way the IPCs will still validate the PCR. However, it cannot validate the integrity of nucleic acid extraction process. Thus it is recommended to add the IPC gene fragments to samples prior to Nucleic acid extraction when circumstances allow.

Reagent preparation and sample loading

To avoid contamination, Nucleic acid extraction and PCR reagent preparation should happen in separate areas.

Do the following in the reagent preparation area:

1. Equilibrate to room temperature: Take out the components in the kit and place at room temperature. Wait for the temperature to equilibrate to room temperature, and mix well for later use.

2. Prepare qPCR working solution: avoid direct light source. Calculate the amount of working solution needed. The calculation goes like this:
Total amount of PreMix needed: N = sample to be tested (n) + positive control (1) + negative control (1) + 0.5 (redundant volume);
Total PreMix volume = 20uL*(N+2.5). *Note, if IPC is not added during Nucleic acid extraction, 0.5uL of IPC should be added to each reaction.

3. Centrifuge: Thoroughly mix the qPCR working solution and centrifuge so the solution stay at bottom of the tube.

4. Dispense: add 20 uL of working solution into each qPCR tube. Move the tubes to sample loading zone.

Do the following in the sample loading area:

5. Load sample and controls: Add extract from samples into qPCR tubes from step 4 above, 5 uL each tube. Add 5 uL positive control into one qPCR tube. Add 5 uL negative control into another qPCR tube. There should be a total of N+2 tubes, N being the total number of samples to be tested.

PCR instrument settings:

1. Fluorescence channel selection:

Channel	Target
FAM	target gene

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

VIC	IPC
Quencher group	None
Passive Reference (if available)	None

For other instruments, refer to the instrument manual.

2. Thermal cycling program:

set reaction volume to 25 uL. Setting details:

Step		Temperature	Reaction time	Cycle
UDG digestion		50°C	5 min	1
Predenaturation		95°C	30 s	1
Amplification	Denaturation	95°C	5 s	45
	Annealing and Extending	50°C	35 s + data collection	

Data analysis

Follow the instrument user guide for raw data analysis. Adjust the curves so the starting values are between 3 to 10 and end values are between 5 to 20.

Results Interpretation

Validation criteria:

The test is valid if the following criteria are met:

Reaction Type	Positive Control	Negative Control
hexon gene/FAM	Ct<=32	Ct>40
IPC/VIC	Ct<=32	Ct>40

Sample data interpretation

Interpretation	Positive	Suspected positive, need to test again	Negative
hexon gene/FAM	Ct<=35	36<=Ct<=40	Ct>40
IPC/VIC	Ct<=35 with clear S-shaped curve	Ct<=35 with clear S-shaped curve	Ct<=35 with clear S-shaped curve

If a sample is suspected to be positive, test again, and if Ct<=40 still, it is deemed positive.

BOSTER BIOLOGICAL TECHNOLOGY
3942 B Valley Ave, Pleasanton, CA 94566
888-466-3604 | support@bosterbio.com | www.bosterbio.com

If the IPC Ct of a sample is >40, the reaction has failed, possible reasons see below:

Possible reasons for assay failure and troubleshooting

1. There are PCR inhibitors in the sample. Solution: dilute the sample before testing.

2. Nucleic acid extraction failed. Solution: Troubleshoot nucleic acid extraction method, extract Nucleic acid again and retest.

3. Samples were not collected properly or had degraded during storage or transportation. Solution: collect new samples.

Note: false negative result could be caused by strain mutations in the target gene.

General Instructions

1) This product is for research use only, please read this manual carefully before use.
2) Be mindful of implementing good quality control for each experiment.
3) qPCR is a highly sensitive assay and is subject to cross contamination. Laboratory personnel should strictly follow the PCR laboratory best practices. The experimental personnel must be professionally trained. The experimental process should be carried out in separate areas as per instruction. The consumables are only for one-time use only. Equipment and instrumentations should not be cross-used.
4) All test samples are regarded as infectious substances, garments and gloves should be changed frequently during the procedure to avoid cross-contamination.
5) Comply with sample handling and waste disposal regulations of your region.
6) All reagents should thaw and mix thoroughly before use.
7) When serum samples are severely hemolyzed, the amplification curve will be significantly lowered. When using this kit to detect serum samples, it is recommended to set different threshold to ensure the accuracy of the results.
8) When there are multiple samples and controls with Ct values >40, it may be the qPCR instrument's default threshold is high. Set the threshold to about 1/15 of the maximum fluorescence value of the amplification curve to get the proper Ct values.