Dog CHV / Canine Herpes Virus IgG antibodies ELISA Kit

Enzyme Immunoassay for the screening of IgG antibody to Canine Herpes Virus (CHV) in dog's serum or plasma.

Catalog number: ARG80989

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

PRINCIPLE OF THE ASSAY

This is an Enzyme immunoassay for the screening of IgG antibody to CHV in dog's serum or plasma.

Purified Canine Herpes Virus (CHV) protein has been pre-coated onto a microtiter plate. Controls, standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
CHV-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Positive Control	1 vial (lyophilized)	4°C.
Negative control	1 vial (lyophilized)	4°C.
HRP-Antibody conjugate	12 ml (Ready-to-use)	4°C
ELISA buffer	18 ml (Ready-to-use)	4°C
200X Wash buffer	20 ml	4°C

Substrate A	8 ml	4°C
Substrate B	8 ml	4°C
STOP solution	8 ml	4°C
Plastic cover seal	1	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 200X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- In ELISAs, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results.

Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.

- Before using the reagents needed, take them out of the kit and place them on the table for ± 15 min at room temperature (± 21 °C) without exposing them to direct sunlight or (other) heat sources.
- Buffer, controls, standards and conjugates need to be shaken gently before use, in order to dissolve/mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or resuspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 200X Wash buffer into distilled water to yield 1X Wash buffer.
- Negative Control: Reconstitute negative control with 1 ml distilled water, divide into aliquots, and store after complete dissolving immediately at -20°C until use (stock solution). Avoid repeated freeze-thaw cycles.
- Positive Control: Reconstitute positive control with 0.5 ml distilled water, divide into aliquots, and store after complete dissolving immediately at -20°C until use. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE (QUALITATIVE)

All materials should be equilibrated to room temperature (RT) before use. Samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it, and use them within 10 days.
2. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (250 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. Take care that none of the wells dry out before the next reagent is dispensed.
3. Negative Control: (for duplicates) Dilute stock solution 1:30 in ELISA buffer to make working solution. Make sure to make minimal 250 µL of every

dilution to be able to transfer 100 µL to the coated plate.

4. Positive Control: (for duplicates) Dilute stock solution 1:30 in ELISA buffer to make working solution. Make sure to make minimal 250 µL of every dilution to be able to transfer 100 µL to the coated plate.
5. Serum or Plasma: (for duplicates) Dilute serum or plasma samples 1:200 in ELISA buffer. Make sure to make minimal 250 µL of every dilution to be able to transfer 100 µL to the coated plate.
6. Take 2 wells as substrate controls, add only 100 µL ELISA buffer to these well.
7. Add 100 µl of diluted samples, positive and negative controls into respective wells.
8. Seal and incubate for 60 minutes at 37°C.
9. Wash as according to step 2.
10. Add 100 µl HRP-antibody conjugate solution into each well. Incubate for 60 minutes at 37°C.
11. Wash as according to step 2.
12. Mix equal part of substrate A and substrate B with gentle shaking. Prepare immediately before use. The mixed substrate can only be used within 1-2 hours after being mixed. Please only prepare amount needed.
13. Add 100 µl of mixed substrate reagent to each well. Incubate for 10-15 minutes at room temperature in dark. Make sure the negative control does not become too dark.
14. Add 50 µl of Stop Solution to each well.
15. Read the OD with a microplate reader at 450 nm immediately. (620 nm as optional reference wave length) and use the substrate controls as blank.

ASSAY PROCEDURE (QUANTITATIVE)

All materials should be equilibrated to room temperature (RT) before use. Samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it, and use them within 10 days.
2. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (250 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. Take care that none of the wells dry out before the next reagent is dispensed.
3. Negative Control: (for duplicates) Make a 3-step dilution of the negative control in ELISA buffer starting with 1:30, 1:90, 1:270 and 1:810 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 250 µL of every dilution to be able to transfer 100 µL to the coated plate.
4. Positive Control: (for duplicates) Make a 3-step dilution of the positive control in ELISA buffer starting with 1:30, 1:90, 1:270 and 1:810 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 250 µL of every dilution to be able to transfer 100 µL to the coated plate.
5. Serum or Plasma: (for duplicates) Make 3-step dilutions of each sample in ELISA buffer starting with 1:30, 1:90, 1:270 and 1:810 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 250 µL of every

dilution to be able to transfer 100 µL to the coated plate.

6. Take 2 wells as substrate controls, add only 100 µL ELISA buffer to these well.
7. Add 100 µl of diluted samples, positive and negative controls into respective wells.
8. Seal and incubate for 60 minutes at 37°C.
9. Wash as according to step 2.
10. Add 100 µl HRP-antibody conjugate solution into each well. Incubate for 60 minutes at 37°C.
11. Wash as according to step 2.
12. Mix equal part of substrate A and substrate B with gentle shaking. Prepare immediately before use. The mixed substrate can only be used within 1-2 hours after being mixed. Please only prepare amount needed.
13. Add 100 µl of mixed substrate reagent to each well. Incubate for 10-15 minutes at room temperature in dark. Make sure the negative control does not become too dark.
14. Add 50 µl of Stop Solution to each well.
15. Read the OD with a microplate reader at 450 nm immediately. (620 nm as optional reference wave length) and use the substrate controls as blank.

VALIDATION OF THE TEST

Qualitative:

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 0.850

- The MV of the measured OD value for the Negative Control (NC) must be ≤ 0.350

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation

Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Quantitative:

In order to confirm appropriate test conditions the OD of the positive control should be ≥ 0.850 OD units (450 nm) and give an endpoint titer of ≥ 90. The negative control should be < 0.350 OD units (450 nm) and give an endpoint titer of ≤ 30.

INTERPRETATION OF TEST RESULTS

Qualitative:

The results are valid if the following criteria are met:

A sample with the S/P ratio < 0.23 is negative

(Specific antibodies to Canine Herpes Virus could not be detected)

A sample with the S/P ratio ≥ 0.23 is positive

(Specific antibodies to Canine Herpes Virus were detected)

Quantitative:

The ELISA titer can be calculated by constructing a curve and using a cut-off line (dilution 1:30 → 1:90→ 1:270→ 1:810→ 1:2430→ 1:7290, etc., total 8 dilutions of 3 steps) OD on Y-axis and titer on X-axis.

ELISA titers can be calculated using as cut-off 2.5 times OD value of negative control at 1:30.