SVANOVIR® EHV1/EHV4-Ab

Equine Herpesvirus type 1 and 4 Discriminating Test

Contents	**Art. No. SV-104901**
Microtitre plate Microtitre plates (96 wells) coated with non-infectious EHV1 antigen (columns 1, 4, 7, 10), EHV4 antigen (columns 2, 5, 8, 11) and control antigen (columns 3, 6, 9, 12) (sealed and stored dry)	2 (Strips) 12 x 8
Conjugate - Concentrate (horseradish peroxidase conjugated rabbit anti-horse IgG antibodies)	1 x 30 µL
PBS-Tween Solution 20 x concentrate	1 x 125 mL
Sample/Conjugate Dilution Buffer Ready-to-use	2 x 30 mL
Substrate Solution (Tetramethylbenzidine in substrate buffer containing H_2O_2) - STORE IN THE DARK!	1 x 20 mL
Stop Solution Contains sulphuric acid (2M) - DANGER! 	1 x 10 mL
A. EHV1/EHV4 Positive Control Serum - Contains preservatives	1 x 2.5 mL
B. EHV1/EHV4 Negative Control Serum - Contains preservatives	1 x 2.5 mL
C. EHV4 Positive Control Serum - Contains preservatives	1 x 2.5 mL

This manual covers the following SVANOVIR® EHV1/EHV4-Ab kit: Article number SV-104901

Equine Herpesvirus type 1 and 4 Discriminating Test

Name and Application

SVANOVIR® EHV1/EHV4-Ab is a enzyme linked immunosorbent assay (ELISA) for the detection of and/or discrimination of specifi antibodies to Equine Herpesvirus type 1 and type 4 in equine serum and plasma samples.

General information

Equine Herpesvirus type 1 and 4 infect horses throughout the world. The nature of the infection differs between the two virus types. EHV4 is the major cause of respiratory disease in young horses while EHV1 is a major cause of abortion. Because polyclonal antibodies to EHV1 and EHV4 are highly cross-reactive, serological determination of the infection caused by either of the two virus types so far has not been possible. This ELISA test discriminates between antibodies to EHV1 and EHV4.

Principle

The kit procedure is based on a solid phase indirect ELISA. In this procedure, samples are added to wells coated with non-infectious EHV1 and EHV4 antigen, and also to a well coated with control antigen (altogether three wells per sample) in microtitre strips. If antibodies directed to EHV1 or EHV4 are present in the test sample, they will bind to the antigens in the wells. The HRP conjugate added subsequently forms a complex with these antibodies. Unbound material is removed by rinsing before the addition of a substrate solution. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate. A positive result is indicated by development of the blue colour. The reaction is stopped by addition of the stop solution; the colour changes to yellow. The result can be read visually or by a microplate photometer, where the optical density (OD) is measured at 450 nm.

Materials needed but not provided

1. Precision pipettes
2. Disposable pipette tips
3. Distilled water, deionised or any similar hight quality water
4. Wash bottle, multichannel pipettor or plate washer
5. Container: 1 to 2 litres for PBS-Tween
6. Microplate photometer, 450 nm fil er
7. Microplate shaker

Specimen information

Undiluted serum or plasma is required for the test. Fresh, refrigerated, previously frozen but *not inactivated* serum or plasma can be used.

Preparation of reagents

PBS-Tween Buffer:

Dilute the PBS-Tween Solution 20 x concentrate 1/20 in distilled water. Prepare 500 mL per plate by adding 25 mL PBS-Tween solution to 475 mL distilled water and mix thoroughly.

N.B. Please check that there is no crystal precipitation in the bottle. If crystals are seen, please warm and shake well.

Conjugate:

Conjugate should be diluted in Sample/ Conjugate Dilution Buffer according to the given dilution factor on the label and on the inner side of the box. It is recommended to prepare a pre-dilution of the conjugate, (for instance 1/100) for further preparation of the final orking dilution.

Pre-dilution of samples:

For testing, the samples should be pre-diluted 1/100 in Sample/Conjugate Dilution Buffer, (for example 5 µL sample into 495 µL of Sample/Conjugate Dilution Buffer).

Precautions

1. Carefully read and follow all instructions.
2. Store the kit and all reagents at 2-8°C (36-46°F).
3. All reagents should equilibrate to room temperature 18-25°C (64-77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction manuals from different test kit batches.
6. Care should be taken to prevent contamination of kit components.
7. Do not use test kit beyond date of expiry.
8. Do not eat, drink, or smoke where specimens or kit reagents are handled.
9. Use a separate pipette tip for each sample.
10. Do not pipette by mouth.
11. Include positive and negative serum controls (3 controls) on each plate or test strip series.
12. Use only distilled, deionised or any similar high quality water for preparation of reagents.
13. When preparing the buffers, etc., measure the required volume.
14. The Stop Solution contains sulphuric acid, which is corrosive.*
15. All unused biological materials should be disposed according to the local, regional and national regulations.

Recommendations!

The volume of the reagents is sufficient or at least 4 separate test occasions.
Strips with broken seal can be stored at 2-8°C (36-46°F) for up to 4 weeks.

Procedure

1. All reagents should equilibrate to room temperature 18-25°C (64-77°F) before use. Label each strip with a number.
2. In duplicates add 100 µL of Positive Control Serum (A and C) and 100 µL of Negative Control Serum (B) to selected wells. For confirmation purp ses it is recommended to run the control solutions in duplicates, (proposed layout, please see fi ure #1).
3. Add 100 µL of prediluted serum sample to each well, (proposed layout, please see fi ure #1).
4. Seal strips and incubate for 2 hours at room temperature 18-25°C (64-77°F) under shaking.
5. Rinse the plates/strips 4 times with PBS-Tween Buffer: at each rinse cycle fill u the wells, empty the plate and tap hard to remove all remains of fluid
6. Add 100 µL of diluted HRP conjugate to each well. Seal the plate and incubate the plate for 1 hour at room temperature 18-25°C (64-77°F) under shaking.
7. Repeat step #5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 10 minutes at room temperature 18-25°C (64-77°F). Begin timing when the fi st well is filled
9. Stop the reaction by adding 50 µL Stop Solution to each well. Add the Stop Solution in the same order as the Substrate Solution in step #8.
10. Measure the optical density (OD) of the controls and samples at 450 nm in a microplate photometer (use air as blank). Measure the OD within 15 minutes after the addition of Stop Solution to prevent flu tuation in OD values.

Figure #1: Recommended process chart for addition of controls and samples

	1	2	3	4	5	6
A	Control A	Control A	Control A	Sample 3	Sample 3	Sample 3
B	Control A	Control A	Control A	Sample 4	Sample 4	Sample 4
C	Control B	Control B	Control B	Sample 5	Sample 5	Sample 5
D	Control B	Control B	Control B	Sample 6	Sample 6	Sample 6
E	Control C	Control C	Control C	Sample 7	Sample 7	Sample 7
F	Control C	Control C	Control C	Sample 8	Sample 8	Sample 8
G	Sample 1	Sample 1	Sample 1	Sample 9	Sample 9	Sample 9
H	Sample 2	Sample 2	Sample 2	Sample 10	Sample 10	Sample 10

Calculations

Corrected OD values (OD_{Corr})

The optical density (OD) values in wells coated with EHV1 and EHV4 are corrected by subtracting the OD values of the corresponding wells containing the control antigen.

$$OD_{EHV1} - OD_{Control\ Antigen} = OD_{Corr\ EHV1}$$

$$OD_{EHV4} - OD_{Control\ Antigen} = OD_{Corr\ EHV4}$$

All controls and samples OD values should be corrected before results are interpretated.
Calculate the mean OD_{Corr} value for each of the controls and samples.

Interpretation of the results

Criteria for test validity

To ensure validity, the duplicate of the OD values of the positive controls should not differ more than 25% from the mean value of the two duplicates.
Additionally, the control values should fall within the following limits;

OD_{Corr} Positive Control > 0.6

OD_{Corr} Negative Control < 0.1

Should any of these criteria not be fulfilled, the test in invalid. For invalid tests, technique may be suspect and the assay should be repeated.

Interpretation of samples

OD > 0.2 Positive

OD 0.1-0.2 Doubtful

OD < 0.1 Negative

Samples with a corrected OD value between 0.1 and 0.2 are considered doubtful and should be retested. If the results are still doubtful, it is recommended to test a second sample from the animal, obtained after a period of 10-14 days.

References

1. B.S Crabb, C.M MacPherson, G.H Reubel, G.F Browning, M.J Studdert, H.E Drummer. A type-specific se ological test to distinguish antibodies to equine herpesviruses 4 and 1. Archive of Virology, 1995, 140: 245-258.
2. H.E Drummer, A Reynolds, M.J Studdert, C.M MacPherson, B.S Crabb. Application of an equine herpesvirus 1 (EHV1) type-specifi ELISA to the management of an outbreak of EHV1 abortion. Veterinary Record, June 10, 1995.
3. A. Nordengrahn, M. Merza, G. Svedlund, M. Ronéus, L. Treiberg Berndtsson, A. Lindholm, H.E Drummer, M.J Studdert, I. Abusugra, E. Gunnarson, B. Klingeborn. A field tudy of the application of a type-specifi test distinguishing antibodies to equine herpesvirus-4 and -1. Equine Infectious Diseases, VIII.

***DANGER: Stop solution (sulphuric acid)**
May be corrosive to metals. Causes skin irritation. Causes serious eye irritation.

Keep only in original container. Wear eye protection/ face protection. Wear protective gloves.
IN CASE OF CONTACT WITH EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician. If eye irritation persists: Get medical advice/ attention.
IN CASE OF CONTACT WITH SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse. If skin irritation occurs: Get medical advice/attention. Absorb spillage to prevent material damage.

Symbols

	Article No.
	Serial (batch) No.
	Temperature limit
	Expiry date
	Number of tests
	See manual
	Manufacturer

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