General Information

This diagnostic kit is designed to detect *Hypoderma bovis and Hypoderma lineatum* specific antibodies.

It can be used with bovine serum or plasma (individual sample or pools up to 10 samples) or milks (individual or bulk milk samples).

Description and Principle

Bovine Hypodermosis is a parasitic disease caused by the development of *Hypoderma bovis* and *Hypoderma lineatum* fly larvae.

In the early stage of the disease, L1 larvae excrete hypodermines A, B and C, to act notably onto the immune system of the host. These antigens become, therefore, among the most immunogenic during infection.

The ID Screen® Hypodermosis Indirect kit allows the detection of the antibodies specific for *Hypoderma bovis* and *H. lineatum* notably thanks to its microplates coated with hypodermins A, B and C from *H. lineatum*.

Samples to be tested and controls are added to the microwells. Anti-hypodermins A/B/C antibodies, if present, form an antibody-antigen complex.

An anti-ruminant horseradish peroxidase (HRP) conjugate is added to the microwells. It binds to the anti-hypodermins A/B/C antibodies, forming an antigen-antibody-conjugate-HRP complex.

After washing in order to eliminate the excess of conjugate, the Substrate Solution (TMB) is added.

The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested:

- in the presence of antibodies, a blue solution appears, which becomes yellow after addition of the Stop Solution.
- in the absence of antibodies, no coloration appears.

The microplate is read at 450 nm.

Kit Components

Reagents*
Microplates coated with hypodermins A, B, and C
Concentrated Conjugate (10X)
Positive Control
Negative Control
Dilution Buffer **2**
Dilution Buffer **3**
Wash Concentrate (20X)
Substrate Solution
Stop Solution (0.5 M)

** Quantities supplied are indicated on the kit label.*

1. The conjugate, controls and substrate solution must be stored at 5°C (± 3°C).
2. The other reagents can be stored between +2°C and +26°C.
3. Wash, substrate and stop solutions can be used for the entire IDvet product range. Dilution buffers with same batch numbers are interchangeable.

Materials required but not provided

1. Mono or multi-channel pipettes capable of delivering volumes of 10 µl, 100 µl, and 300 µl.
2. Disposable tips.
3. 96-well pre-dilution microplate.
4. Distilled or deionized water.
5. Manual or automatic wash system.
6. 96-well microplate reader.

Precautions

1. Do not pipette by mouth.
2. The substrate solution can be irritating to the skin.
3. The stop solution (0.5 M) may be harmful if swallowed. It may cause sensitisation by skin contact (**R22-43**). Avoid contact with skin (**S24-37**).
4. Do not expose the substrate solution to bright light nor to oxidizing agents.
5. All wastes should be properly decontaminated prior to disposal. Dispose in accordance with local regulations.

Sample Preparation

In order to avoid differences in incubation times between specimens, it is possible to prepare a 96-well plate containing the test and control specimens, before transferring them into an ELISA microplate using a multichannel pipette.

Wash Solution Preparation

If necessary, bring the Wash Concentrate **(20X)** to room temperature and mix thoroughly to ensure that the Wash Concentrate is completely solubilized.

Prepare the Wash Solution **(1X)** by diluting the Wash Concentrate **(20X)** in distilled/deionized water.

The quality of the wash step may influence results. Ensure that wells are completely empty between washes. If using an automatic washer, it is extremely important to correctly parameter the machine (mode, type of aspiration, aspiration height). For more information, please consult the "IDvet Washing Guide", available upon request at info@id-vet.com.

Testing Procedure

Allow the reagents to come to room temperature (21°C ± 5°C) before use. Homogenize all reagents by inversion or vortexing.

SERUM OR PLASMA (individual samples or pools of up to 10 samples):

1. Add 90 µl **Dilution Buffer 2** to each microwell.
2. Distribute :
   - 10 µl of the **Negative Control** to wells A1 and B1.
   - 10 µl of the **Positive Control** to wells C1 and D1.
   - 10 µl of each sample to be tested to the remaining wells.
3. Cover the plate and incubate **45 min ± 4 min** at **21°C (± 5°C).**
4. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.

MILK (individual or bulk milk samples):

This test can be performed on skimmed or whole milk samples, with or without preservatives.

When analysing whole milk samples, special washing precautions should be taken (please refer to "Recommendations for milk testing").

Centrifuge each whole milk sample, or just let the samples sit, so that the cream separates from the lactoserum (cream on the top, lactoserum on the bottom). Pipette under the cream so that only the lactoserum enters the cone (antibodies are found in the lactoserum).

1. Pre-dilute **Positive Control** and **Negative Control** at 1:10 in **Dilution Buffer 2**.
2. Distribute :
   - 100 µl of pre-dilute **Negative Control** to wells A1 and B1.
   - 100 µl of pre-dilute **Positive Control** to wells C1 and D1.
   - 100 µl of each milk sample to be tested to the remaining wells.
3. Cover the plate and incubate **45 min ± 4 min** at **21°C (± 5°C).**
4. Empty the wells. Wash each well **5 times** with at least 300 µl of the **Wash Solution.** Avoid drying of the wells between washes. *Be careful that there is no fatty ring left in the well after washing. To avoid fat residues, it is possible to include a soaking time of 2 – 5 minutes between washes.*

FOR ALL PROTOCOLS:

5. Prepare the **Conjugate 1X** by diluting the **Concentrated Conjugate 10X** to 1:10 in **Dilution Buffer 3.**
6. Add 100 µl of the **Conjugate 1X** to each well.
7. Cover the plate and incubate **30 min ± 3 min** at 21°C (± 5°C).
8. Empty the wells. Wash each well 3 times with at least 300 µl of the **Wash Solution**. Avoid drying of the wells between washes.
9. Add 100 µl of the **Substrate Solution** to each well.
10. Cover the plate and incubate **15 min ± 2 min** at 21°C (± 5°C) in the dark.
11. Add 100 µl of the **Stop Solution** to each well, in the same order as in step No.9, to stop the reaction.
12. Read and record the O.D. at 450 nm.

Validation

The test is validated if:

✓ the mean value of the Positive Control optical density (OD_{PC}) is greater than 0.350.

$$\mathbf{OD_{PC} > 0.350}$$

✓ the ratio of the mean values of the Positive to Negative Controls ODs (OD_{PC} to OD_{NC}) is greater than 3.

$$\mathbf{OD_{PC}/OD_{NC} > 3}$$

Interpretation

For each sample, calculate the S/P percentage (S/P%):

$$\boldsymbol{S/P\,\% = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100}$$

SERUM OR PLASMA (individual samples or pools of up to 10 samples):

Samples presenting a S/P%:

- less than or equal to 45% are considered negative.
- between 45% and 55% are considered doubtful.
- greater than or equal to 55% are considered positive.

Result	Status
S/P % ≤ 45 %	NEGATIVE
45% < S/P % < 55 %	DOUBTFUL
S/P % ≥ 55 %	POSITIVE

MILK (individual or bulk milk samples):

Samples presenting a S/P%:

- less than 30% are considered negative.
- greater than or equal to 30% are considered positive.

Result	Status
S/P % < 30 %	NEGATIVE
S/P % ≥ 30 %	POSITIVE

Note: The IDSoft™ data analysis program is available free-of-charge. Please contact **support.software@id-vet.com** for more information.

This software program can calculate many parameters (validation criteria, S/P or S/N values, titers, vaccination age, groups) and offers a graphic representation of the serological profiles of the animals tested).

IDvet
Innovative Diagnostics

Certified management system

ID Screen®
Hypodermosis Indirect

Indirect ELISA for the detection of *Hypoderma bovis* and *H. lineatum* specific antibodies in bovine serum, plasma or milk (individual or pooled samples)

For *in vitro* use

HYPOS ver 0117 EN

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