*Lilli*Test Sheep Scab ELISA Test kit

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An indirect enzyme linked immunosorbent assay (ELISA) to determine the presence of *Psoroptes ovis* antibodies in ovine sera.

1. INTRODUCTION

Sheep scab is caused by infestation of the skin by the non-burrowing ectoparasitic mite *Psoroptes ovis.* It occurs worldwide and is a notifiable disease in some countries. The disease is endemic in the UK and has significant economic impact through its effects on performance and welfare.

2. PRINCIPLE

The *P. ovis* immunoassay is a sensitive, specific and robust diagnostic ELISA for the detection of IgG antibodies specific to the defined recombinant sheep scab mite protein, Pso o 2, in the serum of affected sheep. Animals that have been infested with *P. ovis* produce IgG against Pso o 2. As with any blood test of this type, which measures antibody levels in the blood, it indicates exposure to the parasite but cannot discriminate between whether a sheep has a current infestation or a very recently resolved infestation. The assay is developed and produced by the Moredun Research Institute and globally marketed by Lillidale Diagnostics.

3. MATERIALS SUPPLIED

Material supplied	Qty
Microtitre test plate – 96 well	1
Microtitre test plate sealer	3
Phosphate buffered saline (PBS)	2 sachets
Pso o 2 antigen	15µg
Reference serum: Positive control,ovine serum (C++)	15µl
Reference serum: Negative control,ovine serum (C-)	15µl
Rabbit anti-sheep IgG HRP conjugated antibody	10µl
TMB substrate	5ml
TMB stop solution	5ml

4. MATERIALS REQUIRED BUT NOT INCLUDED

- Single/multi-channel pipettes
- Disposable plastic tips
- ELISA microtitre plate reader and filter for reading absorbance at 450nm
- Distilled/deionised water
- NaCl
- Reagent reservoirs
- Measuring cylinders
- 37ºC incubator
- Timer
- Tween® 20
- Tween® 80

5. PREPARATION OF REAGENTS/ WORKING DILUTIONS AND STORAGE

5.1 WASH BUFFER (WB):

Empty the contents of one sachet of PBS into one litre of distilled/deionised water. Add 500µl (to a final concentration of 0.05% v/v) of Tween®20 and stir with a magnetic stirrer for 10 minutes. Store at 4°C and bring to room temperature (RT) prior to use.

5.2 BLOCKING BUFFER (BB):

Empty the contents of one sachet of PBS into one litre of distilled/deionized water. Add 5mls (to a final concentration of 0.5% v/v) Tween®80 and 29.22g NaCl and stir with a magnetic stirrer for 15 minutes. Store at 4°C and bring to RT prior to use.

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NB: Label and store at 4°C for no longer than one month

5.3 Pso o 2 ANTIGEN

Bring Pso o 2 antigen to RT and vortex to mix. Dilute Pso o 2 in distilled/deionised water to a concentration of 2.5µg/ml to a final volume of 6ml. Store at 4°C.

5.4 REFERENCE SERUM:

Store the reference serum vials (C++ and C-) at -20°C and thaw thoroughly prior to use.

5.5 HRP-LABELLED CONJUGATE:

Dilute the concentrated HRP-labelled conjugate to 1:2,000 in BB.

NB: The concentrated conjugate must be stored in its original vial at 4°C.

5.6 TMB SUBSTRATE:

Ready to use. Store at 4°C and bring to RT prior to use. The substrate solution must be colourless when prepared. If coloured, it must be discarded and a fresh solution must be used. Unused substrate should be discarded and NOT poured back in the vial or saved for later use.

NB: Fresh substrate must be decanted for each test day.

5.7 STOP SOLUTION:

Ready to use. Store at RT.

5.8 TEST SERA:

A clotted blood sample should be centrifuged at 4,000g for 5 minutes, after which the serum can be aspirated and tested.

NB: Before testing, sera should be examined for haemolysis (haemolytic sera would be red in colour), lipemic (whitish in colour), and for contamination (cloudiness). In these cases, the sera must be discarded.

If not tested immediately, test sera should be aspirated from the clot and stored at 4°C for 72 hrs. Longer duration of storage requires serum sample to be frozen at -20°C in appropriately labelled cryopreservation vials.

6. PLATE LAYOUT

C++ Positive reference serum
C- Negative reference serum
1- 40 Test sera in duplicates
BC Blank control

	1	2	3	4	5	6	7	8	9	10	11	12
A	C++	3	7	11	15	19	23	27	31	35	39	BC
B	C++	3	7	11	15	19	23	27	31	35	39	BC
C	C-	4	8	12	16	20	24	28	32	36	40	BC
D	C-	4	8	12	16	20	24	28	32	36	40	BC
E	1	5	9	13	17	21	25	29	33	37	41	C++
F	1	5	9	13	17	21	25	29	33	37	41	C++
G	2	6	10	14	18	22	26	30	34	38	42	C-
H	2	6	10	14	18	22	26	30	34	38	42	C-

7. TEST/ASSAY PROCEDURE

NB: Unless otherwise stated volumes used are 50µl/well, and washes are performed using 300µl of WB/well.

7.1 Add 50µl of diluted Pso o 2 to each well of the ELISA plate and ensure bottom of well is covered by this solution.

7.2 Seal plate with a plate sealer and incubate overnight at RT

7.3 Empty the plate by sharply expelling the contents into a sink

7.4 Blot the plate dry on disposable paper towels

7.5 Wash plate 5 times

7.6 Add 100µl of BB per well

7.7 Re-seal plate and incubate for 60 min at 37°C

7.8 Repeat steps 7.3-7.5 then proceed to step 7.9

7.9 Dilute the test sample sera and C++ and C- control sera 1/400 v/v in BB

7.10 Add 50ul of each sample to each of 2 wells and 50ul of control sera to each of 4 wells (C++ and C-). Add 50ul BB to each blank well according to the template.

7.11 Re-seal plate and incubate for 60 min at 37°C

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7.12 Repeat steps 7.3-7.5 ensuring WB is retained in wells for at least 1 minute during washes 4 and 5 then proceed to step 7.13.

7.13 Dilute rabbit anti-sheep IgG HRP conjugate 1/2000 v/v in BB

7.14 Add 50µl of diluted conjugate to each well

7.15 Re-seal plate and incubate for 60 min at 37°C

7.16 Bring TMB substrate solution to RT

7.17 Repeat steps 7.3-7.5 ensuring WB is retained in wells for at least 1 minute during washes 4 and 5 then proceed to step 7.18.

7.18 Add 50µl of TMB substrate to all wells; incubate at RT for 10 minutes (do not seal).

7.19 Add 50µl of TMB Stop solution to each well

7.20 Read the optical density at 450nm using an ELISA microtitre plate reader

8. ASSAY PERFORMANCE AND INTERPRETATION OF RESULTS

Measure the OD of all wells at 450nm within 10-15 minutes after colour development has been stopped.

8.1 VALIDATION CRITERIA:

Calculate the means for the C++ and C- control sera wells and for the Blank wells across the plate.
The test is valid if the following criteria are met:

- The C++ positive control is within the OD450nm range: 1.8-2.2
- The C- negative control OD450nm is not >0.25
- The mean OD450nm of the blank wells is <0.04

8.2 INTERPRETATION OF OD450nm DATA:

≥0.5 = test positive*
0.4-0.49 = suspicion of positivity (re-test)
≤0.39 = test negative

*For the purposes of diagnosis, a positive result in the ELISA alone should not be interpreted as evidence of active infestation with *P. ovis* in the absence of clinical examination by a veterinary surgeon.

9. QUALITY CONTROL

It is good laboratory practice to plan your tests in advance. Check that all equipment is available and working, and that all reagents and control and test sera are prepared and thawed if necessary.

If reference serum is to be repeatedly used, aliquot into smaller volumes upon delivery to prevent multiple freeze-thaw cycles.

Where possible it is good practice to always include at least 3 blank wells each time a plate is run, regardless of the number of sera samples being processed on the plate.

Care should be taken to ensure the accurate measurement of reagents by using well calibrated pipettes. For each pipetting operation, new and clean disposable tips must be used.

Care must be taken to ensure the high quality of the distilled/deionized water used for the preparation of your reagents and buffer solutions.

Only immuno-plates provided with the kit should be used for the test and they are not reusable.

Do not use plate washers for wash steps, manual washing is advised. The plate reader should be turned on and allowed to warm up for at least 15 minutes prior to reading the test plate. This warm up period is necessary to ensure the uniformity of reading for all test plates.

Before reading the test plate, ensure there are no bubbles in any of the wells, as this will cause optical aberrations. Ensure that there is no condensation or smudges (e.g. finger prints) on the bottom of the test plate. If necessary, rupture any bubbles using a clean pipette tip and wipe the bottom of the plate clean with a soft cloth.

10. ORDERING INFORMATION

*Lilli*Test Sheep Scab ELISA Test kit, 1 plate, 46 tests, Product code #VE-3004

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