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SERELISA™ ParaTB Mono Indirect

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50282000
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MYCOBACTERIUM PARATUBERCULOSIS ANTIBODY TEST KIT

In-vitro diagnostic kit for the detection of Mycobacterium avium paratuberculosis (ParaTB) in bovine serum and plasma and caprine serum.

GENERAL INFORMATION AND INTENDED USES

SERELISA™ ParaTB Ab Mono Indirect use an immunoenzymatic technique for the detection of antibodies to ParaTB in bovine serum or plasma, and caprine serum samples. The principle of the test is as follows: Samples obtained from cattle or goats within herds exposed to ParaTB contain specific anti-ParaTB antibodies. Samples diluted in Sample Diluent (containing Mycobacterium phlei extracts) are added to antigen coated wells. Specific antibody in the serum forms an antibody-antigen complex with the antigen bound to the plate. After washing the plate, a monoclonal anti-bovine IgG HRP peroxidase conjugate is added to each well. The antibody-antigen complex remaining binds with the conjugate. After a brief incubation period, the unbound conjugate is removed by a second wash step. Substrate, which contains a chromogen (ABTS), is added to each well. Chromogen color change (from clear to green-blue) occurs in the presence of the peroxidase enzyme. After the substrate has incubated, Stop Solution is added to each well to terminate the reaction and the plate is read using an ELISA plate reader at 405-410 nm. Comparison of the assay results from unknown samples with those of known positive and negative controls provides the basis for determination of sample status in comparison to a sample/positive (S/P) cutoff.

KIT COMPOSITION AND CONSERVATION

This SERELISA™ ParaTB Kit contains materials sufficient to test a maximum of 460 samples.

<table>
<thead>
<tr>
<th>ITEM</th>
<th>REAGENT NATURE</th>
<th>VOLUME</th>
<th>RECONSTITUTION AND CONSERVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5 microplates containing 12 strips of 1 X 8 wells coated with M. a. paratuberculosis antigen</td>
<td>5 x 96 wells</td>
<td>Remaining wells may be used for up to 3 months after the pouch is first opened, provided the pouch is resealed and stored at 2°C – 7°C.</td>
</tr>
<tr>
<td>SD</td>
<td>Sample Diluent; preserved with phenol and gentamicin sulfate</td>
<td>1 x 100 mL</td>
<td>Ready to use</td>
</tr>
<tr>
<td>CONTROL</td>
<td>40X Negative control; preserved with Microcide III</td>
<td>1 x 200 μL</td>
<td>Dilute to 1X in Sample Diluent. Use within 8 hours of dilution.</td>
</tr>
<tr>
<td>CONTROL</td>
<td>40X Positive control</td>
<td>1 x 200 μL</td>
<td>Dilute to 1X in Sample Diluent. Use within 8 hours of dilution.</td>
</tr>
<tr>
<td>C</td>
<td>100X HRP Conjugate</td>
<td>1 x 800 μL</td>
<td>Dilute to 1X in Conjugate Diluent. Use within 2 hours of dilution.</td>
</tr>
<tr>
<td>CD</td>
<td>Conjugate Diluent; preserved with phenol and gentamicin sulfate</td>
<td>1 x 75 mL</td>
<td>Ready to use</td>
</tr>
<tr>
<td>W</td>
<td>20X Wash Solution; preserved with Imidazole</td>
<td>1 x 200 mL</td>
<td>Dilute to 1X in deionized or reverse osmosis water. Diluted Wash Solution can be stored at 23°C ± 5°C and used for up to 3 months following dilution.</td>
</tr>
<tr>
<td>ABTS</td>
<td>Substrate</td>
<td>1 x 100 mL</td>
<td>Ready to use</td>
</tr>
<tr>
<td>S</td>
<td>5X Stop Solution (5% SDS)</td>
<td>1 x 25 mL</td>
<td>Dilute to 1X in deionized or reverse osmosis water. Diluted Stop Solution can be stored at 23°C ± 5°C and used for up to 3 months following dilution.</td>
</tr>
</tbody>
</table>

Note: All reagents provided in the kit should be stored at 2°C - 7°C. Reagents should not be frozen.
REAGENTS REQUIRED TO PERFORM 92 TESTS*

a) 1 ParaTB Antigen coated microplate
b) 20 mL Sample Diluent
c) 10 μL 40X Negative Control
d) 10 μL 40X Positive Control
e) 110 μL 100X HRP Conjugate
f) 11 mL Conjugate Diluent
g) 20 mL 20X Wash
h) 11 mL ABTS Substrate
i) 2.5 mL 5X Stop

* Volumes required are applicable when 92 samples are run within one single plate.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

a) High precision multiple delivery pipetting devices (i.e. 1-20 μL. Measurement deviation must be ≤ 10 % for volumes ≤ 10 μL and ≤ 5 % for all other volumes)
b) 0.2 mL, 1.0 mL, and 5.0 mL pipettes and tips
c) 8 or 12 channel pipettes (or automated device)
d) 2 graduated cylinders (100 mL and 1000 mL)
e) 1 mL or 5 mL borosilicate glass test tubes
f) Uncoated low binding 96 well microplates
g) Deionized or reverse osmosis water
h) Microplate reader with 405-410 nm filter
i) Microplate washing apparatus
j) Waste container with bleach or other oxidizing agent

WARNINGS TO THE USERS OF REAGENTS AND ANTIGEN COATED MICROPLATES

• Handle all reagents and samples as biohazardous material. It is recommended to dispose reagents and contaminated material according to the applicable regulations.
• Wear suitable protective clothing.
• Take care not to contaminate any test reagents with samples or bacterial agents.
• If the humidity indicator of a microplate exhibits a pink color, the microplate should not be used.
• The best results are achieved by following the protocols as they are described below, using good, safe laboratory techniques.
• Never add water to the microplates, conjugate, controls, or substrate.
• Do not use this kit after the expiration date.
• NEVER PIPETTE BY MOUTH. Harmful if swallowed.
• For veterinary use only.

Refer to the end of this insert for reagent hazard and precaution statements. Also reference the Safety Data Sheet for additional details.

NOTE: STORE ALL REAGENTS PROVIDED IN THE KIT AT 2 – 7 °C. REAGENTS SHOULD NOT BE FROZEN. ALLOW COMPONENTS TO COME TO TEMPERATURE (23 °C ± 5 °C) FOR AT LEAST ONE HOUR BEFORE STARTING.

SAMPLE COLLECTION

• Follow proper sample collection procedures.
• Harvest and store properly:
  • serum and plasma up to four days at 2 °C – 7 °C or -20 °C for longer.
  • Test only good quality samples (i.e. avoid bacterial contamination, heavy hemolysis or lipemia). When in doubt, obtain a better quality sample.

SERELISA® ParaTB Ab Mono Indirect


SAMPLE AND CONTROL DILUTION PROCEDURE
Dilute samples using the Sample Diluent provided in a clean, uncoated 96 well microplate (Sample Dilution Microplate) or using vials or microfuge tubes. Samples should be completely thawed and thoroughly mixed before diluting. The following table describes the sample and control dilution procedure using a sample dilution microplate.

<table>
<thead>
<tr>
<th>STEP</th>
<th>UNITS</th>
<th>MATERIAL</th>
<th>LOCATION</th>
<th>FINAL DILUTION</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>195 µL</td>
<td>Sample Diluent</td>
<td>Each well</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>2)</td>
<td>5 µL</td>
<td>Sample</td>
<td>Add into all wells excluding the control wells (A1, A2, B1, B2)</td>
<td>1:40</td>
<td>Mix well. Discard pipette tips after each sample.</td>
</tr>
<tr>
<td>3)</td>
<td>5 µL</td>
<td>40X Negative Control</td>
<td>Into wells A1 and A2</td>
<td>1:40</td>
<td>Mix well.</td>
</tr>
<tr>
<td>4)</td>
<td>5 µL</td>
<td>40X Positive Control</td>
<td>Into wells B1 and B2</td>
<td>1:40</td>
<td>Mix well.</td>
</tr>
<tr>
<td>5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Allow all diluted controls and samples to equilibrate for 5 minutes before transferring to the ELISA microplate.</td>
</tr>
</tbody>
</table>

PREPARATION OF 1X CONJUGATE, 1X WASH AND 1X STOP SOLUTIONS

<table>
<thead>
<tr>
<th>STEP</th>
<th>UNITS</th>
<th>MATERIAL</th>
<th>LOCATION</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>6)</td>
<td>10.89 mL</td>
<td>Conjugate Diluent</td>
<td>Clean test tube or bottle</td>
<td>Mix well. 1:100 final dilution.</td>
</tr>
<tr>
<td>7)</td>
<td>110 µL</td>
<td>100X Conjugate</td>
<td>Mix well.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STEP</th>
<th>UNITS</th>
<th>MATERIAL</th>
<th>LOCATION</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>8)</td>
<td>20 mL</td>
<td>20X Wash</td>
<td>Microplate washing bottle or apparatus</td>
<td>Mix well. 1:20 final dilution</td>
</tr>
<tr>
<td>9)</td>
<td>380 mL</td>
<td>Deionized or reverse osmosis water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>STEP</th>
<th>UNITS</th>
<th>MATERIAL</th>
<th>LOCATION</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>10)</td>
<td>2.5 mL</td>
<td>5X Stop</td>
<td>Clean tube or bottle</td>
<td>Warm 5X Stop Solution to room temperature or to 37 °C and mix to dissolve any precipitates.</td>
</tr>
<tr>
<td>11)</td>
<td>10 mL</td>
<td>Deionized or reverse osmosis water</td>
<td>Mix well. 1:5 final dilution</td>
<td></td>
</tr>
</tbody>
</table>

ELISA TEST PROCEDURE

<table>
<thead>
<tr>
<th>STEP</th>
<th>UNITS</th>
<th>MATERIAL</th>
<th>LOCATION</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>a)</td>
<td></td>
<td></td>
<td></td>
<td>Remove the test microplate (or the required number of strips if whole microplate is not needed) from the protective bag. Return any unused wells to a pouch with desiccant and properly seal for storage.</td>
</tr>
<tr>
<td>b)</td>
<td>100 µL</td>
<td>Diluted controls and samples (Step 5)</td>
<td>Add into appropriate microplate wells</td>
<td>Change pipette tips for each new sample</td>
</tr>
<tr>
<td>c)</td>
<td></td>
<td>Incubate microplate for 30 minutes (± 5 min) at 23 °C ± 5 °C.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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WASH PROCEDURE

<table>
<thead>
<tr>
<th>STEP</th>
<th>UNITS</th>
<th>MATERIAL</th>
<th>LOCATION</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>d)</td>
<td></td>
<td>Either manually or with an automated washer, discard or aspirate solution from all wells into an appropriate waste container.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>e)</td>
<td>300 µL</td>
<td>Diluted Wash Solution (Steps 8-9)</td>
<td>Each test well</td>
<td>Wash process is a critical step for an ELISA. Please follow steps d through i.</td>
</tr>
<tr>
<td>f)</td>
<td></td>
<td>Allow Wash solution to soak in wells for 10-15 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>g)</td>
<td></td>
<td>Discard or aspirate solution from all wells into an appropriate waste container.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>h)</td>
<td>Repeat wash procedure (steps e-g) 2 more times.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i)</td>
<td></td>
<td>Invert and blot onto absorbent pad to remove remaining liquid after final wash.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ADDITION OF 1X CONJUGATE, SUBSTRATE, AND 1X STOP SOLUTION

<table>
<thead>
<tr>
<th>STEP</th>
<th>UNITS</th>
<th>MATERIAL</th>
<th>LOCATION</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>j)</td>
<td>100 µL</td>
<td>1X Conjugate Solution (steps 6-7)</td>
<td>Each test well</td>
<td>Discard pipette tips.</td>
</tr>
<tr>
<td>k)</td>
<td></td>
<td>Incubate for 30 minutes (± 5 min) at 23 °C ± 5 °C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l)</td>
<td></td>
<td>Follow the WASH PROCEDURE above (steps d to i).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>m)</td>
<td>100 µL</td>
<td>Substrate</td>
<td>Each test well</td>
<td>Discard pipette tips.</td>
</tr>
<tr>
<td>n)</td>
<td></td>
<td>Incubate for 15 minutes (± 1 min) at 23 °C ± 5 °C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o)</td>
<td>100 µL</td>
<td>1X Stop Solution (Steps 10-11)</td>
<td>Each test well</td>
<td>Discard pipette tips.</td>
</tr>
<tr>
<td>p)</td>
<td></td>
<td>Gently tap to mix contents within the wells. Read the microplate using an ELISA microplate reader set at a single wavelength of 405 or 410 nm. Allow bubbles to dissipate before reading.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

ASSAY CONTROL VALUES, VALID ELISA RESULTS:
Negative Control average optical density (OD) is ≤ 0.25.
Positive Control average OD is between 0.40 and 1.20.
If either value is out of range, the test results should be considered invalid and the samples should be retested.

MANUAL PROCESSING OF DATA

a) Calculate the average Negative Control absorbance optical density (OD) using the values of wells A1 and A2. Calculate the average Positive Control OD using values obtained from wells B1 and B2. Record both averages.
b) Subtract the average Negative Control OD from the average Positive Control OD. The difference is the Corrected Positive Control.
c) Calculate a sample to Positive (S/P) ratio by subtracting the average Negative Control OD from each sample OD and dividing the difference by the corrected Positive Control. Use the following equation format:

\[
S/P = \frac{\text{SAMPLE OD} - \text{AVERAGE NEGATIVE CONTROL OD}}{\text{CORRECTED POSITIVE CONTROL}}
\]

SERELISA® ParaTB Ab Mono Indirect
EXAMPLE:

Example Positive Control ODs: 0.585 and 0.605
Average = (0.585 + 0.605) / 2 = 0.595

Corrected Positive Control: (0.595) – (0.067) = 0.528

Example Negative Control ODs: 0.072 and 0.062
Average = (0.072 + 0.062) / 2 = 0.067

Example S/P value calculation:
OD of sample = 0.560
[(0.560) – (0.067)] / 0.528 = 0.934

INTERPRETATION OF RESULTS

The SERELISA™ ParaTB Ab Mono Indirect S/P values should be interpreted using the following ranges:

For bovine serum or plasma and caprine serum:

<table>
<thead>
<tr>
<th>S/P 0.70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum or plasma sample</td>
</tr>
</tbody>
</table>

a) Negative: Samples with S/P value < 0.70 are presumed negative for antibody.

b) Positive: Samples with a ParaTB S/P ratio value ≥ 0.70 are presumed positive for antibody.

NOTE:

A variety of factors, such as possible variations that may exhibit atypical biological and/or antigenic properties, prevalence of ParaTB within a herd and timing and randomness of sample collection procedures could result in an infected herd yielding negative ELISA results. It is therefore recommended that each herd only be considered to be negative after each herd has been adequately sampled and repeatedly tested several times and has yielded negative results each time by ELISA or standard conventional methods (culture, PCR, etc.). Additional confirmation testing of samples collected from presumed ELISA antibody positive herds, using standard techniques, are needed to confirm a positive diagnosis of a ParaTB infection within a herd.