SERELISA™ BVDV Erns Ag Capture

Zoetis Inc.
16420 Via Esprillo
San Diego, CA 92127

Zoetis Inc.
Kalamazoo, MI 49007, USA
VLN/PCN 190/5A05.22
1-888-963-8471
www.zoetis.com

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BOVINE VIRUS DIARRHEA ANTIGEN TEST KIT

In-vitro diagnostic kit for the detection of BVDV Erns antigen in bovine ear tissue.

GENERAL INFORMATION AND INTENDED USES

SERELISA™ BVDV Erns Ag Capture uses an immunoenzymatic technique for the detection of Erns antigen from both type 1 and type 2 Bovine Viral Diarrhea Virus (BVDV), which allows the identification of PI animals. The assay is performed on ear notch samples which have been soaked in extraction buffer. The individual, processed ear notch sample extracts can be applied directly to wells coated with anti-Erns antibody (mixture of type 1 specific and type 2 specific monoclonal antibodies). In positive samples, Erns antigen extracted from ear notch samples will form an antibody-antigen complex with the anti-Erns antibody bound to the plate. After washing the plate, another set of monoclonal anti-Erns antibodies conjugated with Horseradish peroxidase (HRP) is added to each well. The antibody-antigen complex remaining from the previous step reacts with the conjugate. After a 15 minute incubation period, the unbound conjugate is removed by a second wash step. Subsequently, substrate, which contains a chromogen (TMB), is added to each well. A chromogenic color change (from clear to blue) occurs in the presence of the peroxidase enzyme. After incubation with the substrate, stop solution is added to each well to inhibit the reaction and spectrophotometric values are recorded using an ELISA plate reader set at a single wavelength of 630 or 650 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™ BVDV Kit contains materials sufficient to test a maximum of 460 samples.

ITEM	REAGENT NATURE	VOLUME	RECONSTITUTION AND CONSERVATION
A	5 microplates containing 12 strips of 1 X 8 wells coated with anti-BVDV Erns monoclonal antibodies	5 X 96 wells	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 – 7 °C.
CONTROL -	Negative control; preserved with sodium azide	1 X 6 mL	Ready to use
CONTROL +	Positive control; preserved with bromonitrodioxane	1 X 6 mL	Ready to use
C	100X HRP-Conjugate; preserved with bromonitrodioxane	1 X 650 µL	Dilute to 1X in Conjugate Diluent. Use within 2 hours of dilution.
CD	Conjugate diluent; preserved with bromonitrodioxane	1 X 65 mL	Ready to use (Blue color)
W	20X Wash; preserved with Imidazole	1 X 200 mL	Dilute to 1X in deionized or reverse osmosis water. Diluted Wash Solution can be stored at 18 - 28 °C and used for up to 3 months following dilution.
TMB	Substrate	1 X 65 mL	Ready to use
S	5X Stop (5% SDS)	1 X 25 mL	Dilute to 1X in deionized or reverse osmosis water. Diluted Stop Solution can be stored at 18 - 28 °C and used for up to 3 months following dilution.
E	10X Extraction buffer; preserved with sodium azide	1 X 100 mL	Dilute to 1X in deionized or reverse osmosis water. Diluted Extraction buffer can be stored at 18 - 28 °C and used for up to 3 months following dilution.
	Adhesive films	25 films	Ready to use

Store all reagents provided in the kit at 2 – 7 °C. Reagents should not be frozen.

REAGENTS REQUIRED TO PERFORM 92 TESTS

a) 1 Anti-BVDV Erns antibody coated microplate
b) 200 µL Negative Control
c) 200 µL Positive Control
d) 110 µL 100X Conjugate
e) 11 mL Conjugate Diluent
f) 20 mL 20X Wash
g) 11 mL Substrate
h) 2.5 mL 5X Stop
i) 100 mL 10X Extraction Buffer

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

a) High precision multiple delivery pipetting devices (i.e. 1-20 and 20-200 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) Disposable tubes for soaking ear notch samples: 5 mL tubes for large ear notches, 1.5 mL centrifuge tubes for small ear notch samples
f) Deionized or reverse osmosis water
g) Microplate reader with 630 or 650 nm filter
h) Microplate washing apparatus (manual or automated)
i) Waste container for disposal of reagents and contaminated materials in accordance with applicable regulations
j) If additional 1X Extraction Buffer is required then it can be formulated as follows:

To manufacture 1 Liter of 1X Extraction buffer

1. In 900 mL of deionized or reverse osmosis water add:
   0.26 g Sodium Phosphate Monobasic ($NaH_2PO_4 \cdot H_2O$);
   1.15 g Sodium Phosphate Dibasic (Na_2HPO_4);
   8.75 g Sodium Chloride (NaCl);
   adjust if needed to acceptable pH range of 7.4 ± 0.2.
2. Add 10 mL of Triton X-100, 1 g Sodium Azide
3. Add water to 1 Liter final volume.
4. Store solution when not in use at room temperature for up to 3 months.

WARNINGS TO THE USERS OF REAGENTS AND ANTIBODY COATED PLATES

- 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 described below, using good, safe laboratory techniques.
- Never add water to the antibody coated 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 (18 – 28 °C) FOR AT LEAST ONE HOUR BEFORE STARTING.

SAMPLE COLLECTION

Before collecting the ear notch, dip the notching tool in disinfectant and then rinse away residual disinfectant with copious quantities of clean water. Obtain a proper ear notch size from a clean portion of the ear. For short term storage, ear notches may be stored at 2 –7 °C. If refrigerated storage is not available, ear notches may be stored dry at 15 – 30 °C for up to 3 days. For long term storage, the ear notch samples should be kept frozen (-20 °C or colder).

SAMPLE PREPARATION

a) Prepare 1X Extraction Buffer by diluting 10X Extraction Buffer in laboratory grade (deionized or reverse osmosis) water. For example, dilute 10 mL 10X Extraction Buffer in 90 mL water (1:10). Mix well. Diluted Extraction Buffer solution may be stored at room temperature for up to 3 months.
b) Add the required volume of Extraction Buffer Solution (see Table 1) to the disposable tubes and follow the instructions in Table 1 for inversion, incubation time, and sample storage.

TABLE 1. SAMPLE PREPARATION:

	LARGE EAR NOTCH	SMALL EAR NOTCH
SIZE REQUIREMENT	Ear notch is at least 1 cm on one side	Ear notch is at least 2-3 mm in diameter and <1 cm on all dimensions
1X EXTRACTION BUFFER SOLUTION	1.5 mL	0.5 mL
MIX BY INVERSION	4 times or by vortexing	4 times or by vortexing
INCUBATION TIME	At least **10 minutes** (range: 10 minutes - 1 hour)	At least **1 hour** (range: 1 hour - 3 days)
SAMPLE STORAGE	If the processed ear notch sample extract will not be tested immediately, it should be stored at 2 – 7 °C for up to 48 hours. For prolonged storage, the extract should be kept frozen (-20 °C or colder).	

SAMPLE DILUTION PROCEDURE

The individual processed ear notch sample extract does not require dilution prior to addition to the antibody coated plate. If the processed ear notch sample extract is frozen, it should be completely thawed and thoroughly mixed prior to assay.

PREPARATION OF CONTROLS

NO DILUTION OF THE BVDV POSITIVE AND NEGATIVE CONTROLS IS NEEDED.

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

STEP	UNITS	MATERIAL	LOCATION	NOTES
1X CONJUGATE SOLUTION				
1)	10.89 mL	Conjugate Diluent	Clean tube or bottle	Mix well. 1:100 final dilution.
2)	110 µL	100X Conjugate		
1X WASH SOLUTION				
3)	20 mL	20X Wash	Microplate washing bottle or apparatus	Mix well. 1:20 final dilution.
4)	380 mL	Deionized or reverse osmosis water		
1X STOP SOLUTION				
5)	2.5 mL	5X Stop	Clean tube or bottle	Warm 5X Stop at room temperature or 37 °C and mix to dissolve any precipitates from storage.
6)	10 mL	Deionized or reverse osmosis water		Mix well. 1:5 final dilution

ELISA TEST PROCEDURE

STEP	UNITS	MATERIAL	LOCATION	NOTES
a)	Remove the antibody coated test microplate (or the required number of strips if whole plate is not needed) from the protective bag.			
b)	100 µL	Individual processed ear notch sample extract	Add into each test microplate well excluding control wells	Change pipette tips for each new sample
c)	100 µL	Negative control	A1 and A2	Discard pipette tip.
d)	100 µL	Positive control	B1 and B2	Discard pipette tip.
e)	Incubate microplate for 60 minutes* (± 5 min) at 23 ± 5 °C.			

* **Optional overnight sample incubation protocol:**

- After sample and controls have been added to the microplate, cover the wells with adhesive film which has been cut to the proper length for the number of strips used.
- Incubate the microplate overnight (14-18 hours) at 2 –7 °C.
- The next morning, allow components (including the microplate) to come to 23 ± 5 °C for at least one hour.
- Then proceed to the **WASH PROCEDURE**.

WASH PROCEDURE

f)	Either manually or with an automated washer, discard or aspirate solution from all wells into an appropriate waste container			
g)	300 µL	1X Wash Solution (steps 3 - 4)	Each test well	**Wash process is a critical step for an ELISA. Please follow steps f to i. Tap inverted to dry.**
h)	Discard contents into an appropriate waste container			
i)	Repeat wash procedure (Steps g through h) 3 more times.			
j)	Invert and blot onto absorbent pad to remove remaining liquid after final wash			

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

k)	100 µL	1X Conjugate Solution (steps 1 - 2)	Each test well	Discard pipette tips.
l)	Incubate for 30 minutes (± 5 min) at 23 ± 5 °C.			
m)	Follow the **WASH PROCEDURE** above (steps f to j).			
n)	100 µL	Substrate	Each test well	Discard pipette tips.
o)	Incubate 15 minutes (± 1 min) at 23 ± 5 °C.			
p)	100 µL	1X Stop Solution (steps 5 - 6)	Each test well	Discard pipette tips.
q)	Read the microplate using an ELISA microplate reader set at a single wavelength of 630 or 650 nm. Allow bubbles to dissipate before reading.			

RESULTS

ASSAY CONTROL VALUES, VALID ELISA RESULTS:

Negative Control average optical density (OD) is < 0.150. Average Positive Control OD is between 0.200 and 0.800. 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 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:

$$\text{S/P} = \frac{\text{(SAMPLE OD) - (AVERAGE NEGATIVE CONTROL OD)}}{\text{CORRECTED POSITIVE CONTROL}}$$

EXAMPLE:

Example Positive Control ODs:
0.303 and 0.332
Average = (0.303 + 0.332) / 2 = 0.318

Corrected Positive Control:
(0.318) – (0.046) = 0.272

Example Negative Control ODs:
0.040 and 0.052
Average = (0.040 + 0.052) / 2 = 0.046

Example S/P value calculation:
OD of sample = 0.560
[(0.560) – (0.046)] / 0.272 = 1.890

INTERPRETATION OF RESULTS

SERELISA® BVDV Erns Ag Capture S/P ratios obtained for ear notch samples should be interpreted as follows:

BVDV Presumed Antigen Status:

a) Negative: Ear notch samples with S/P ratios of < 0.15 are presumed negative for BVDV antigen.
b) Positive: Ear notch samples with S/P ratios ≥ 0.15 are presumed positive for BVDV antigen. A positive result may not definitively indicate the animal is persistently infected (PI); the animal might be acutely infected with BVDV. The same animal should be retested after three weeks to confirm its PI status. If the result remains positive, the animal will be identified as PI animal.*

***NOTE:** Due to the high concentration of antigen present in some positive ear notch samples, OD values may be close to 4.0 and exceed the upper limit of some plate readers. If this situation is encountered, then provided the controls are valid and the sample well intensity is dark, the sample should be presumed positive for antigen to BVDV. As for other presumed positive results, adhere to the guidance given in the above section "Interpretation of Results" for appropriate follow-up. If additional guidance is desired, contact Zoetis.