

**UBI® FMDV NS 3B NEUTRALIZATION BUFFER**  
**For**  
**UBI® FMDV NS 3B NEUTRALIZATION ASSAY (SWINE)**  
**or**  
**UBI® FMDV NS 3B NEUTRALIZATION ASSAY (CATTLE)**

DIRECTION INSERT

FOR INVESTIGATIONAL USE ONLY  
(P/N 100165)

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U.S. Patent No. 6,048,538  
Other Patents Worldwide

## **TRADE NAME & INTENDED USE**

UBI<sup>®</sup> FMDV NS NEUTRALIZATION BUFFER is used in conjunction with the UBI<sup>®</sup> FMDV NS ELISA (SWINE) or the UBI<sup>®</sup> FMDV NS ELISA (CATTLE) for the confirmatory testing of samples that are repeatably reactive by the UBI<sup>®</sup> FMDV NS ELISA (SWINE) or the UBI<sup>®</sup> FMDV NS ELISA (CATTLE)

## **SUMMARY AND EXPLANATION OF THE NEUTRALIZATION ASSAY**

Foot-and-Mouth Disease Virus (FMDV) is the causative agent of Foot-and-Mouth Disease (FMD) in cloven-hoofed animals. The virus is highly contagious and is controlled by vaccination, quarantine, and the slaughter of affected herds. Diagnosis is by clinical observation of lesions in the mouth and on the legs, with confirmation by virus isolation. However, rapid diagnosis by symptoms alone can be confounded because the symptoms of FMD can resemble those of other diseases such as vesicular stomatitis and swine vesicular disease. Moreover, convalescent asymptomatic animals may serve as persistent carriers. Therefore, diagnosis and surveillance of FMDV infection is aided by serological tests.

FMDV occurs as seven antigenically distinct serotypes: A, O, C, Asia 1, and SAT 1, 2, 3 whose structural proteins frequently do not display extensive serologic cross-reactivity. Non-structural proteins are more conserved and can serve as antigens for the detection of group-specific antibodies. Non-structural proteins also can be useful as differential antigens to distinguish between immuno-reactivities associated with infection and immunoreactivities that result from vaccination. However, these proteins, as long polypeptide antigens, may not differentiate infected from vaccinated animals because the inactivated viral extracts used in vaccines frequently contain sufficient amounts of the non-structural proteins to provoke antibody responses in vaccinated animals.

The UBI<sup>®</sup> FMDV NS ELISA (SWINE) and UBI<sup>®</sup> FMDV NS ELISA (CATTLE) are enzyme-linked immunosorbent assays (ELISAs) which employ a synthetic peptide as the solid-phase immunosorbent for detection of antibodies to infectious FMDV. The peptide that constitutes this sensitizing agent contains antigenic determinants taken from immunoreactive domains of non-structural (NS) FMDV protein 3B. The short site-specific synthetic peptide of the UBI<sup>®</sup> FMDV NS ELISA does not possess the serological cross-reactivities that have been observed for longer NS polypeptide antigens derived from virus or recombinant organisms. The use of synthetic peptides minimizes the incidence of non-specific reactions originating from antibody reactivities in the specimen towards host cell antigens or expression vector antigens which are co-purified with virus-derived or recombinant-antigens.

The existence of false positive samples can impose a serious problem for large-scale surveillance programs, as swine or cattle herds may be misidentified as infected, and farmers or breeders may be wrongfully penalized for harboring infected animals. Thus, easy-to-perform confirmatory neutralization assays for immunoassays such as the UBI<sup>®</sup> FMDV NS ELISA are useful to reduce the rate of serum samples that are falsely repeatably reactive during surveys of normal herds.

## **CHEMICAL AND BIOLOGICAL PRINCIPLES OF THE PROCEDURE**

UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER is designed to test whether a positive seroreactivity identified by a UBI<sup>®</sup> FMDV NS ELISA results from the specific reaction of serum antibodies to the NS 3B peptide. A 3B seroreactivity specifically directed to FMDV will be markedly reduced by mixing the serum with NS 3B NEUTRALIZATION BUFFER containing the NS 3B peptide. In contrast, a non-specific 3B seroreactivity will be largely unaffected by mixing the sample with NS 3B NEUTRALIZATION BUFFER.

During the course of the 3B Neutralization Assay, each specimen is diluted in duplicate; in one well, the specimen is mixed and diluted with the Specimen Diluent of the UBI<sup>®</sup> FMDV NS ELISA (SWINE) or (CATTLE), and in the other well, it is mixed with the NS 3B NEUTRALIZATION BUFFER. The diluted samples are then added to the REACTION MICROPLATE wells. FMDV NS 3B-specific antibodies, if present, bind with the liquid-phase 3B peptide antigen during mixing with the Neutralization Buffer, and most of them are no longer available for binding to the solid-phase 3B peptide coated on the REACTION

MICROPLATE. Non-specific antibodies do not bind to the solution-phase peptide antigen after mixing with the Neutralization Buffer, and remain capable of binding to the solid-phase 3B peptide coated on the REACTION MICROPLATE. Any specimen showing a reduction of  $A_{450\text{nm}}$  of 40% or greater in the FMDV NS ELISA following mixing with 3B Neutralization Buffer ( $\geq 40\%$  Neutralization Rate) is considered seropositive (i.e., contains 3B-specific antibodies). Any specimen showing a Neutralization Rate less than 40% is considered negative for antibodies to FMDV.

## REAGENT COMPOSITION AND STORAGE CONDITIONS

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### FMDV NS 3B NEUTRALIZATION BUFFER

20 mL

Buffered salt solution containing synthetic FMDV NS 3B peptide, carrier proteins and preservatives: 0.02% gentamycin and 0.1% sodium azide.

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## MATERIALS REQUIRED - NOT SUPPLIED

1. UBI<sup>®</sup> FMDV NS 3B ELISA (SWINE) or UBI<sup>®</sup> FMDV NS 3B ELISA (CATTLE) kit.
2. Adjustable single channel micropipette to deliver 10  $\mu\text{L}$  to 200  $\mu\text{L}$  with at least 5% accuracy, or equivalent pipettor diluter.
3. Incubator ( $37^\circ \pm 2^\circ\text{C}$ ).
4. Polypropylene tubes or containers (25 mL capacity) with screw top caps or equivalent.
5. Sodium hypochlorite solution, 5.25% (liquid household bleach), or an iodophor as disinfectant.
6. The UBI<sup>®</sup> FMDV NS ELISA test procedure requires a microplate reader capable of transmitting light at a wavelength of 450 nm.
7. Aspiration-wash system capable of dispensing and aspirating 250-350  $\mu\text{L}$ .
8. Pipettor troughs or boats.
9. Reagent grade (USP or equivalent) water.
10. Disposable gloves.
11. 10 mL serological pipettes.
12. Timer.
13. Absorbent tissue.
14. Biohazardous waste containers.
15. Disposable pipet tips.

## STORAGE OF REAGENTS

1. Opened, unused UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER in the original container is stable through the expiration dating indicated on its label.
2. Store the UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER at  $2^\circ$  to  $8^\circ\text{C}$  when not in use.
3. The UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER should be used at room temperature ( $15^\circ$  to  $30^\circ\text{C}$ ).
4. Refer to the Direction Insert of UBI<sup>®</sup> FMDV NS 3B ELISA (SWINE) or UBI<sup>®</sup> FMDV NS 3B ELISA (CATTLE) for reagent storage.

## WARNINGS AND PRECAUTIONS

1. FOR *IN VITRO* DIAGNOSTIC USE ONLY.
2. Must be used only on swine or cattle serum samples in combination with the UBI<sup>®</sup> FMDV NS 3B ELISA (SWINE) or (CATTLE) kits.
3. HANDLE ASSAY SPECIMENS, REACTIVE AND NON-REACTIVE CONTROLS AS IF CAPABLE OF TRANSMITTING AN INFECTIOUS AGENT. Wear disposable gloves and other appropriate protective garb throughout the test procedure. Dispose of gloves and garb as biohazardous waste. Wash hands and laboratory ware thoroughly.
4. Kit components of a UBI<sup>®</sup> FMDV NS 3B ELISA are matched for optimal performance. Do not use interchangeably between kits of different lot numbers. Only use reagents supplied by UBI<sup>®</sup>.
5. Do not use the UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER or components of a UBI<sup>®</sup> FMDV NS 3B ELISA kit beyond the expiration date shown on the labels.
6. Follow the installation, operation, calibration and maintenance instructions provided by the instrument manufacturers for both microplate reader, and the microplate washer.
7. Spills should be wiped up thoroughly using either an iodophor disinfectant or sodium hypochlorite (bleach) solution. Inactivation of acid-containing spills is not required.  
Iodophor Disinfectant: Should be used at a dilution providing at least 100 ppm available iodine.  
Sodium Hypochlorite: Should be used as a 5.25% sodium hypochlorite solution (liquid household bleach).  
Acid-containing spills: Should be wiped dry. Spill areas should then be wiped with a 5.25% sodium hypochlorite solution.  
All materials used to contain and clean up spills should be considered biohazardous and disposed of accordingly.
8. The UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER contains sodium azide as a preservative. Sodium azide has been reported to form lead or copper azides in laboratory plumbing. These azides may explode on percussion, such as hammering. To prevent formation of lead or copper azide, thoroughly flush drains with water after disposing of solutions containing sodium azide. To remove contamination from old drains suspected of azide accumulation, the National Institute for Occupational Safety and Health (USA) recommends the following:
  - (1) siphon liquid from drain trap using a rubber or plastic hose,
  - (2) fill with 10% sodium hydroxide solution,
  - (3) allow to stand for 16 hours, and
  - (4) flush well with water.
9. Refer to the Direction Insert of the UBI<sup>®</sup> FMDV NS 3B ELISA (SWINE) or (CATTLE) for other warnings and precautions.

## WASTE DISPOSAL

Dispose of all specimens and materials used to perform the test as if they contain infectious agents. Dispose of all waste in accordance with all local, state and federal regulations. The preferred method of disposal is autoclaving for a minimum of one half hour at 121°C with 15 lbs pressure. Disposable materials may be incinerated.

Liquid wastes **not containing acid** may be mixed with sodium hypochlorite in volumes such that the final mixture contains 0.5% sodium hypochlorite. Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite. Allow 30 minutes for decontamination to be completed.

## SPECIMEN HANDLING AND PREPARATION

1. Specimens containing precipitates or particulate matter may give inconsistent test results. If necessary, specimens should be clarified by centrifugation prior to testing.
2. If specimens are to be stored, they should be refrigerated at 2° to 8°C or frozen. Multiple freeze-thaw cycles should be avoided.
3. If specimens are shipped, they should be packaged in compliance with national regulations covering the transportation of etiologic agents.

## INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Changes in the physical appearance of the Neutralization Buffer supplied may indicate deterioration. Do **not** use Neutralization Buffer which is visibly turbid or which has a rancid odor.

## ASSAY PROCEDURE

Allow at least 30 minutes for the UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER, and the pouched REACTION MICROPLATE wells and other reagents of the UBI<sup>®</sup> FMDV NS ELISA (SWINE) or (CATTLE) to reach room temperature (15° to 30°C), but do not expose to direct sunlight or elevated temperatures (>30°C).

**Caution:** Verify that dispensing equipment delivers specified sample and/or reagent volumes and does not introduce cross-contamination.

1. Separate dilution microplates are required for specimen dilution. (see the Pipetting Scheme illustrated below).
  - A. Use two wells (A1 and A2) for NON-REACTIVE CONTROL (NRC).
  - B. Use two wells (B1 and B2) for FMDV NS REACTIVE CONTROL (RC).
  - C. Use two wells for each specimen.
  - D. Place the dilution microplates over a microwell locator label.
  - E. Dilute the CONTROLS and SPECIMENS as follows (1:21):
    - a. Add 200 µL of SPECIMEN DILUENT (SD) into each NRC and RC well of the dilution microplate, and into each remaining well of the odd-number columns of the REACTION MICROPLATE where one set of the samples will be mixed and diluted.
    - b. Add 200 µL of UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER (NB) into the remaining wells of column 2 and into the wells of the even-number columns of the REACTION MICROPLATE where one set of the samples will be mixed and diluted.
    - c. Dispense 10 µL of CONTROLS or SPECIMENS into the assigned wells of the dilution microplate. Mix thoroughly by pipetting up and down 3 times. Use a separate pipette tip for each diluted CONTROL or SPECIMEN.

## Pipetting Scheme for Sample Dilution

	1	2	3	4	5	6	7	8	9	10	11	12
A	NR	NR	SD	NB	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-
B	C	C	-7	-7	15	15	23	23	31	31	39	39
C	RC	RC	SD	NB	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-
D			-8	-8	16	16	24	24	32	32	40	40
E	SD	NB	SD	NB	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-
F	-1	-1	-9	-9	17	17	25	25	33	33	41	41
G	SD	NB	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-
H	-2	-2	10	10	18	18	26	26	34	34	42	42
	SD	NB	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-
	-3	-3	11	11	19	19	27	27	35	35	43	43
	SD	NB	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-
	-4	-4	12	12	20	20	28	28	36	36	44	44
	SD	NB	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-
	-5	-5	13	13	21	21	29	29	37	37	45	45
	SD	NB	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-	SD-	NB-
	-6	-6	14	14	22	22	30	30	38	38	46	46

- Cut open the foil pouch and remove the REACTION MICROPLATE. If necessary, remove any excess antigen-coated strips from the frame and return them to the foil pouch. Seal the extra strips securely inside the foil pouch making sure to include the desiccant provided. Return to storage at 2° to 8°C immediately. It may be necessary to replace these strips with blank strips (not provided in the kit), depending on the washing system used.
- Transfer 100 µL of the diluted CONTROLS and SPECIMENS from each well of the DILUTION MICROPLATE to its corresponding well in the REACTION MICROPLATE. Dispose of the DILUTION MICROPLATE and unused liquid as biohazardous waste.
- Within 30 minutes of SPECIMEN/CONTROL addition, cover with the enclosed lid or equivalent and incubate for 60 ± 5 minutes at 37° ± 2°C.
- Prepare WORKING CONJUGATE SOLUTION, as described in the PREPARATION OF REAGENTS section of UBI® FMDV NS 3B ELISA (SWINE) or (CATTLE) prior to washing the microplates.
- Uncover the REACTION MICROPLATE and wash with diluted WASH BUFFER. Discard liquid as biohazardous waste.
  - Automatic Microplate Washer - Use six (6) washes with at least 300 µL/well/wash. It is recommended that the plate be reoriented in the washer following the first three (3) wash cycles, by turning 180 degrees to assure uniform treatment.
  - Manual Microplate Washer - Wash six (6) times, using at least 300 µL/well/wash. Fill the entire plate, then aspirate in the same order.
  - Handheld Multichannel (8 or 12) Pipettor - Wash six (6) times using at least 300 µL/well/wash.
- After washing is complete, excess liquid may be removed from the REACTION MICROPLATE by inverting and tapping on absorbent paper until no further moisture appears on the paper. Dispose of the absorbent paper as biohazardous waste.
- Add 100 µL of the WORKING CONJUGATE solution (1:101) to all wells of the REACTION MICROPLATE.
- Cover and incubate for 30 ± 2 minutes at 37 ± 2°C.

10. Uncover and repeat the wash procedure as in steps 6 and 7.
11. Add 100  $\mu\text{L}$  of TMB SUBSTRATE SOLUTION to each well of the REACTION MICROPLATE.
12. Cover and incubate in the dark for  $15 \pm 1$  minute at  $37 \pm 2^\circ\text{C}$ .
13. Uncover and add 100  $\mu\text{L}$  of STOP SOLUTION to each well of the REACTION MICROPLATE. Mix by gently tapping the side of the REACTION MICROPLATE.
14. Read the absorbance of each well at 450 nm. Blank on air. If a dual filter instrument is used, the reference wavelength should be within the range of 620 to 690 nm.

**NOTE:** Absorbance should be read within 15 minutes of the addition of the STOP SOLUTION to the REACTION MICROPLATE.

15. Review absorbance values for the CONTROLS. Refer to CALCULATION OF RESULTS for control validation parameters and calculation of the NEUTRALIZATION RATE.

## CALCULATION OF RESULTS

### 1. Criteria for Valid Assay Run

- A. The mean absorbance value of the NON-REACTIVE CONTROL (NRC) is  $\leq 0.2$  in the absence of mixing with the 3B Neutralization Buffer. If the mean of the NRC is not within this range, the assay is invalid and must be repeated.
- B. Ensure that the REACTIVE CONTROL values (RC) are  $\geq 0.7$  and  $\leq 1.9$  in the absence of mixing with the 3B Neutralization Buffer. If individual RC values are not within this range, the assay is invalid. The instrument may need to be recalibrated and the tests must be repeated.

### 2. Calculation of Results

#### A. Determine the Mean of NRC.

Example: Sample No.	Absorbance
A1	0.102
A2	0.113
Total	0.215
Mean	$0.215/2 = 0.108$ (NRC)

#### B. Determine the Mean of RC.

Example: Sample No.	Absorbance
B1	1.245
B2	1.191
Total	2.436
Mean	$2.436/2 = 1.218$ (RC)

### C. Calculation of the NEUTRALIZATION RATE

Use the following formula to calculate the NEUTRALIZATION RATE:

#### **% NEUTRALIZATION RATE (NR)**

$$= \frac{(A_{450} \text{ of SD}) - (A_{450} \text{ of NB})}{(A_{450} \text{ of SD})} \times 100\%$$

Where “**A<sub>450</sub> of SD**” is the absorbance of sample mixed with the Specimen Diluent; “**A<sub>450</sub> of NB**” is the absorbance of sample mixed with the NS 3B Neutralization Buffer

Example:

$$A_{450} \text{ of SD} = 0.329$$
$$A_{450} \text{ of NB} = 0.062$$
$$\% \text{ NR} = [(0.329 - 0.062) / 0.329] \times 100\% = 81.2\%$$

### **INTERPRETATION OF RESULTS**

1. Specimens having %NR of less than 40% by the Neutralization Buffer are considered negative for antibodies to infectious FMDV. The reactivity identified by a UBI<sup>®</sup> FMDV NS ELISA is most likely due to non-specific reaction of serum antibodies to the Reaction Microplate. Further testing is not required.
2. Specimens having %NR of equal or more than 40% are considered initially positive for presence of antibodies to infectious FMDV. These specimens should be retested in duplicate (using the original specimen) before final confirmation of the result. (Note: for initial or follow-up tests, the reliability of the tests is increased by testing every sample in at least two replicate wells.)
3. Initially reactive specimens, which do not react in either of the duplicate repeat tests are considered negative for antibodies to FMDV. Further testing is not required.
4. Initially reactive specimens, which are reactive in either of the repeat tests, are considered repeatably reactive for antibodies to FMDV.
5. Repeatably reactive specimens, especially the ones collected from animals having multiple rounds of FMDV vaccinations, are recommended to be further confirmed by a serologically independent confirmatory test: the UBI<sup>®</sup> FMDV NS 3A ELISA (SWINE) or the UBI<sup>®</sup> FMDV NS 3A ELISA (CATTLE).

### **LIMITATIONS OF THE PROCEDURE**

1. The UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER is not valid for use in assays other than the UBI<sup>®</sup> FMDV NS 3B (SWINE) ELISA or the UBI<sup>®</sup> FMDV NS 3B (CATTLE) ELISA.
2. The UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER is not recommended for assays using serum diluted beyond the 1:21 dilution required for optimal assay performance, and for assays using plasma or other bodily fluids. Likewise, pooled and diluted specimens are not recommended for use in this assay.
3. The FMDV NS 3B Neutralization Assay PROCEDURE and the INTERPRETATION OF RESULTS sections must be closely adhered to when testing for the presence of antibodies to infectious FMDV in serum from individual animals.
4. The UBI<sup>®</sup> FMDV NS 3B NEUTRALIZATION BUFFER and a FMDV NS 3B NEUTRALIZATION ASSAY cannot be used to evaluate protective immune responses resulting from vaccination.

The FMDV NS 3B NEUTRALIZATION ASSAY is recommended for use as a tool for confirmation of repeatably reactive samples identified in a UBI<sup>®</sup> FMDV NS ELISA, and is not recommended for screening herds.

UBI® is a registered trademark of United Biomedical, Inc.

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Worldwide patents pending

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