Zorba-NS® Sample Diluent

IVD

REF FA025

CE

INTENDED USE

Zorba-NS® Sample Diluent has been developed and optimized for use as a sample diluent with several of the ZEUS IFA Test Systems. The formulation of this product is intended to reduce the nonspecific fluorescence associated with some serum specimens and thereby improve the readability of these specimens (qualitative interpretation), without affecting the endpoint titers of specific immunofluorescent reactions (quantitative interpretations).

SIGNIFICANCE AND BACKGROUND

Nonspecific fluorescence results from the non-immunological attachment of immunoglobulins to a substrate (fixed cells, microorganisms, tissue sections, etc.), followed by the binding of conjugate. The intensity and distribution (nuclear, cytoplasmic, etc.) of nonspecific staining may vary from sample to sample, the dilution of the sample used in the test procedure, and the composition of the substrate. Some serum specimens produce little or no nonspecific fluorescence. Other specimens produce so much nonspecific fluorescence that a specific staining pattern, if present, will be obscured. When this happens, the sample must be reported as uninterpretable. Alternatively, the sample may be titered to see if a specific pattern can be unmasked within the dilution series.

PRINCIPLE OF THE PROCEDURE

Zorba-NS® Sample Diluent is supplied ready for use, and may be used in place of PBS for the preparation of screening dilutions**. NOTE: Use Zorba-NS® Sample Diluent for screening dilutions only. Do not prepare serial dilutions for endpoint titers in Zorba-NS® Sample Diluent.** Refer to the Procedure section of the respective ZEUS Package Insert and dilute the patient sample using Zorba-NS® Sample Diluent instead of PBS. If the patient requires titering, only the screening dilution should be prepared in Zorba-NS® Sample Diluent. All subsequent two-fold dilutions must be prepared using PBS.

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Zorba-NS® Sample Diluent contains Sodium Azide as a preservative which may be toxic if ingested and has been reported to form lead or copper azide in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of this solution.
3. Remove only the amount of Zorba®-NS Sample Diluent needed to perform each test run to reduce the possibility of product contamination.
4. Zorba® -NS Sample Diluent should be used only as a diluent for patient specimens:
5. Do not use Zorba®-NS Sample Diluent to dilute the Controls or Conjugate.
6. Do not use Zorba-NS® Sample Diluent in any of the wash steps.
7. The volume of Zorba-NS® Sample Diluent supplied has been calculated to provide sufficient material for use with the ZEUS IFA Test Systems when used according to the instructions herein. The use of larger volumes for sample preparation will result in an insufficient amount to allow each test well to be utilized.
8. Zorba-NS® Sample Diluent should only be usedfor IgG testing. For IgM testing, treat with Zorba® IgG Removal Reagent (ZEUS Product #: FA003G).

MATERIALS PROVIDED

DIL	SPE	Zorba-NS® Sample Diluent: Two, 25mL, bottles containing a PBS buffer with proprietary additives to reduce non-specific staining.

STORAGE CONDITIONS

2°C – 8°C	Zorba-NS® Sample Diluent

INTERPRETATION OF RESULTS

Refer to the Interpretation of Results section of the appropriate ZEUS Package Insert.

PERFORMANCE CHARACTERISTICS

In a study conducted by ZEUS Scientific, the performance of the below listed ZEUS IFA Test Systems with Zorba-NS® Sample Diluent was compared to the same Test System using PBS as the Sample Diluent (standard methodology).

1. ZEUS IFA ANA HEp-2 Test System

A study was performed using 206 samples obtained from a plasma donor center and a reference laboratory. The ZEUS IFA ANA HEp-2 Test System with Zorba-NS® Sample Diluent was compared to a commercial ANA HEp-2 Test System (PBS Sample Diluent) and the ZEUS IFA ANA HEp-2 Test System (PBS Sample Diluent). With respect to positive and negative results, there was 96% agreement (198/206) between the ZEUS IFA ANA HEp-2 Test System with Zorba-NS® Sample Diluent and the ZEUS IFA ANA HEp-2 Test System with PBS. In addition, there was 96% agreement (198/206) between the ZEUS IFA ANA HEp-2 Test System with Zorba-NS® Sample Diluent and the commercial ANA HEp-2 Test System with PBS. The discrepant results involved 15 different samples. All 15 were borderline positive when screened using PBS as the Sample Diluent and negative when screened using Zorba-NS® Sample Diluent. One sample was positive on both tests that used PBS as the Sample Diluent. Seven samples were positive using the ZEUS IFA ANA HEp-2 Test System with PBS. The other 7 samples were positive on the commercial ANA HEp-2 with PBS diluent. The discrepant samples were retested at 1:40 and titered in the ZEUS IFA ANA HEp-2 Test System (PBS) and the commercial ANA HEp-2 (PBS) Test System. All 15 samples were again borderline reactive at 1:40, but negative at 1:80. Seventy-nine samples were positive by all three assays with no discrepancies in staining patterns. Twenty-five of the positive samples were used for an endpoint titer analysis. The endpoint titer results are as follows:

	ZEUS IFA ANA HEp-2 Test System (Zorba-NS® Sample Diluent)	
	ZEUS IFA ANA HEp-2 Test System (PBS)	Commercial ANA HEp-2 Test System
Identical Titer	17	5
± One, two-fold dilution	8	13
± Two, two-fold dilutions	0	7
Totals	25	25

2. ZEUS IFA ANA Rat Liver Tissue Test System

Thirty-nine (39) samples, positive for ANA using the HEp-2 substrate, were tested as described above. There was 100% agreement between ZEUS IFA ANA Rat Liver Tissue Test System using Zorba-NS® Sample Diluent (36/39), and ZEUS IFA ANA Rat Liver Tissue Test System using PBS (36/39) with no discrepancies in staining patterns. Sixteen (16) of the Rat Liver positive samples, representing various ANA reactivities, were used for endpoint titer analysis. Fifteen (15) of the 16 samples exhibited identical endpoint titers. The remaining sample was within one, two-fold dilution. Several samples, negative for ANA but positive for anti-mitochondrial, anti-reticulin, anti-smoothmuscle, and anti-bile canaliculus were also evaluated. Zorba-NS® Sample Diluent had no effect on the detection or endpoint titer of these specimens. Seventy-four (74) samples from a normal donor population were tested as described above. Seventy-one (71) of 74 samples (96%) were negative when tested in PBS; 72/74 samples (97%) were negative when tested in Zorba-NS® Sample Diluent. The 3 specimens positive in PBS included the 2 positives in Zorba-NS® Sample Diluent. Only 1 of the 2 samples positive in the ZEUS IFA ANA Rat Liver Tissue Test System using Zorba-NS® Sample Diluent and one of the 3 samples positive in the ZEUS IFA ANA Rat Liver Tissue Test System using PBS (same sample) was positive using HEp-2 substrate. This sample exhibited anti-nuclear envelope reactivity. The 1 sample from the plasma donor population which was positive in PBS and negative when diluted in Zorba-NS® Sample Diluent was borderline reactive at 1:20 and negative at 1:40.

3. **ZEUS IFA ANA Mouse Kidney Tissue Test System**
Thirty-seven (37) samples, positive for ANA using the HEp-2 substrate were tested as described above. There was 100% agreement between the ZEUS IFA ANA Mouse Kidney Tissue Test System using Zorba-NS® Sample Diluent (33/37), and the ZEUS IFA ANA Mouse Kidney Tissue Test System using PBS (33/37) with no discrepancies in staining patterns. Thirteen (13) of the Mouse Kidney positive samples, representing various ANA reactivities, were used for endpoint titer analysis. Nine (9) of the 13 samples exhibited identical endpoint; the remaining 4 samples were ± one, two-fold dilution. Several samples, negative for ANA but positive for anti-smoothmuscle and anti-mitochondrial antibodies were also evaluated. Zorba-NS® Sample Diluent had no effect on the detection or endpoint titer of these specimens. Seventy-four (74) samples from a normal plasma donor population were tested as described above. Sixty-six (66) of 74 samples (89%) were negative when tested in PBS; 71/74 (96%) were negative when tested in Zorba-NS® Sample Diluent. The 8 specimens that were positive in PBS included the 3 positives in Zorba-NS® Sample Diluent. Only 1 of the 3 samples positive in Mouse Kidney using Zorba-NS® Sample Diluent and 1 of 8 samples positive in Mouse Kidney using PBS (same sample) were positive using HEp-2 substrate. This sample exhibited anti-nuclear envelope reactivity. The 5 samples from the plasma donor population which were positive in PBS and negative when diluted in Zorba-NS® Sample Diluent were borderline reactive at 1:20 and negative at 1:40.

4. **ZEUS IFA Autoantibody Screen (AAS) Test System**
Thirty-one (31) samples, positive for ANA using the HEp-2 substrate were tested as described above. There was 100% agreement between the ZEUS IFA Autoantibody Screen (AAS) Test System using Zorba-NS® Sample Diluent (29/31) and the ZEUS IFA Autoantibody Screen (AAS) Test System using PBS (29/31) with no discrepancies in staining patterns. Thirteen (13) of the AAS positive samples, representing various ANA reactivities, were used for endpoint titer analysis. Nine (9) of the 13 samples exhibited identical endpoints; the remaining 4 samples were ± one, two-fold dilution. Several samples, negative for ANA but positive for anti-smoothmuscle, anti-mitochondrial, anti-parietal cell, and anti-reticulin antibodies were also evaluated. Zorba-NS® Sample Diluent had no effect on the detection or endpoint titer of these specimens, regardless of the antibody specificity. Seventy-four (74) samples from a normal plasma donor population were tested as described above. Seventy-three (73) of 74 (98.6%) were negative for ANA when tested in PBS and in Zorba-NS® Sample Diluent. The 1 sample positive in both sample diluents exhibited anti-nuclear envelope reactivity. Thirteen (13) samples from the normal donor population exhibited borderline (1+ at 1:20) reactions in PBS consistent with anti-smoothmuscle (8), anti-reticulin (3) and anti-parietal cell (2) reactivities. The same reactivities were obtained when the samples were diluted in Zorba-NS® Sample Diluent.

5. **ZEUS IFA EBV-VCA Test System**
Seventy-four (74) samples from a normal plasma donor population were tested as described. All 74 samples were positive in PBS and Zorba-NS® Sample Diluent. Forty-seven (47) samples from a pediatric population were obtained from a hospital in the Northeast. Twenty-five (25) of 47 samples were positive in PBS; the same samples were positive when diluted in Zorba-NS® Sample Diluent. The remaining 22 samples were negative when tested in both sample diluents. Ten (10) samples with endpoint titers ranging from 1:40 to 1:1280 in PBS were also titered in Zorba-NS® Sample Diluent. All 10 samples had identical endpoints in the two diluents.

6. **ZEUS IFA EBV-EA Test System**
Forty-four (44) samples, positive for EBV-EA antibody when screened in PBS were also positive when screened in Zorba-NS® Sample Diluent. Ten (10) of these samples, with EBV-EA titers ranging from 1:10 to 1:1280 in PBS had identical endpoint titers when tested in Zorba-NS® Sample Diluent. Thirty (30) samples, negative for EBV-EA antibody when screened in PBS were also screened in Zorba-NS® Sample Diluent. Twenty-nine (29) of the 30 samples were negative in Zorba-NS® Sample Diluent. The 1 discrepant sample was a 1+ positive in Zorba-NS® Sample Diluent; the reaction in PBS was borderline and partially masked by nonspecific fluorescence.

7. **ZEUS IFA Cytomegalovirus (CMV) Test System**
Seventy-four (74) samples from a normal plasma donor population were tested as described. There was 100% agreement between the two diluents; 43 samples were positive and 31 samples were negative in both diluents. Sixteen (16) positive samples with endpoint titers ranging from 1:16 to 1:1024 in PBS were also tested in Zorba-NS® Sample Diluent. Fifteen (15) of 16 samples exhibited identical endpoints in the two diluents; one sample was ± one, two-fold dilution.

8. **ZEUS IFA HSV-1/HSV-2 Test System**
Seventy-four (74) samples from a normal plasma donor population were tested as described above. Forty-nine (49) samples were positive for antibody to HSV-1 when screened at 1:10 in PBS. The same samples were positive when screened in Zorba-NS® Sample Diluent. Ten (10) samples with endpoint titers ranging from 1:10 to 1:2560 in PBS were also titered in Zorba-NS® Sample Diluent. Identical endpoint titers were obtained in each diluent. Twenty-five samples were negative for antibody to HSV-1 when screened in PBS. Twenty-four of 25 samples were also negative when diluted (25) in Zorba-NS® Sample Diluent. The 1 discrepant sample was a 1+ reactive in Zorba-NS® Sample Diluent; the reaction in PBS was borderline and partially obscured by nonspecific fluorescence. When tested for antibodies to HSV-2, 67 samples were positive for antibody to HSV-2 when screened at 1:10 in PBS. The same samples were positive when screened in Zorba-NS® Sample Diluent. Twelve (12) samples with endpoint titers ranging from 1:10 to 1:2560 in PBS were also titered in Zorba-NS® Sample Diluent. Nine (9) of the 12 samples exhibited identical endpoints. The remaining 3 samples were within ± one, two-fold dilution. Seven (7) samples were negative for antibody to HSV-2 when screened in PBS. The same samples were negative when diluted in Zorba-NS® Sample Diluent.

9. **ZEUS IFA Measles IgG Test System**
Seventy-four (74) samples from a normal plasma donor population were tested as described. Sixty (60) samples were positive for Measles IgG when screened at 1:10 in PBS. The same samples were positive when screened in Zorba-NS® Sample Diluent. Eleven samples with endpoint titers ranging from 1:20 to 1:1280 in PBS were also titered in Zorba-NS® Sample Diluent. Ten (10) of 11 samples exhibited identical endpoints. The remaining sample was within ± one, two-fold dilution. Fourteen (14) samples were negative for IgG antibody to measles virus when screened in PBS. The same samples were negative when diluted in Zorba-NS® Sample Diluent.

10. **ZEUS IFA Varicella-Zoster Virus (VZV) Test System**
Seventy-three (73) samples from a normal plasma donor population were tested as described. Seventy-one (71) samples were positive for VZV antibodies when screened at 1:10 in PBS. The same samples were positive when screened in Zorba-NS® Sample Diluent. Eleven (11) samples with endpoint titers ranging from 1:20 to 1:640 in PBS were also titered in Zorba-NS® Sample Diluent. Identical endpoint titers were obtained in each diluent. Two samples (2/73) were negative for antibody to VZV when screened in PBS. Both samples were negative when diluted in Zorba-NS® Sample Diluent. To obtain more information on specificity, 46 pediatric/adolescent samples were screened in PBS and Zorba-NS® Sample Diluent. Twenty-nine (29) specimens were positive in both diluents, and 17 specimens were negative in both diluents.

11. **ZEUS IFA Toxoplasma Test System**
Seventy-four (74) samples from a normal plasma donor population were tested as described above. There was 100% agreement between PBS and Zorba-NS® Sample Diluent. Sixteen (16) of 74 samples (22%) were positive using PBS as sample diluent. The same samples were positive when tested in Zorba-NS® Sample Diluent. The remaining 58 samples were negative when tested in both sample diluents. The 16 positive samples were used for endpoint titer analysis. Fifteen (15) of the 16 samples had identical endpoint titers. The endpoint for the remaining sample was within one, two-fold dilution for the two diluents.

ZEUS Scientific

ZEUS Scientific, Inc.
200 Evans Way, Branchburg, New Jersey, 08876, USA
Toll Free (U.S.): 1-800-286-2111, Option 2
International: +1 908-526-3744
Fax: +1 908-526-2058
Website: www.zeusscientific.com

For US Customer Service contact your local distributor.
For US Technical Support contact ZEUS Scientific, call toll free or e-mail support@zeusscientific.com.
For Non-US Customer Service and Technical Support inquiries, please contact your local distributor.

EC REP
EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

(Rev. Date 12/29/2017)