Zorba® IgG Removal Reagent

REF FA003G

IVD

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INTENDED USE

Zorba® IgG Removal Reagent is designed to functionally remove potentially interfering immunoglobulin G (IgG) antibodies from human serum prior to testing for IgM. The product is for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

The early and accurate diagnosis of many infectious diseases has become increasingly dependent upon the measurement of pathogen-specific IgM antibodies in human serum (1, 2, 3, and 4). IgM antibodies generally appear 7 - 10 days after primary infection, peak in 2 - 3 weeks, and decline to undetectable levels in 3 - 6 months. Therefore, the identification of pathogen-specific IgM in human serum suggests a current or recent infection. In addition, since maternal IgM does not cross the placenta under normal physiological conditions, the presence of pathogen-specific IgM in cord blood or neonatal serum is highly suggestive of a congenital infection.

The identification of pathogen-specific IgM is contingent upon the removal of pathogen-specific IgG for accuracy. Specific IgG in a patient specimen may compete with specific IgM for antigenic sites on the substrate. Depending upon the ratio of IgG to IgM, false negative IgM results may be obtained. In addition, RF-IgM (IgM class anti-IgG) if present in conjunction with specific IgG, may interact with antigen bound IgG resulting in a false positive IgM test. Therefore, the accurate measurement of pathogen-specific IgM is highly dependent upon the effective removal or neutralization of specific IgG.

Removal of pathogen-specific IgG and RF-IgM is most easily accomplished by treating the patient specimen with species (normally goat) anti-human IgG. This treatment will result in the formation of immune complexes containing human IgG, effectively reducing the ability of the pathogen-specific IgG to interact with the substrate. In addition, the aggregated IgG contained in the immune complexes provides an excellent substrate for RF-IgM binding. Therefore, the treated sample will be free of specific IgG and RF-IgM, and can be used for specific IgM measurements.

PRINCIPLE OF THE PROCEDURE

Patient specimens to be tested for pathogen-specific IgM are first diluted in Zorba® IgG Removal Reagent (goat anti-human IgG). Immune complexes containing patient IgG are formed during the subsequent incubation period. RF-IgM, if present, will bind to the immune complexes resulting in the removal of both pathogen-specific IgG and RF-IgM.

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Shake well before using.
3. Zorba® IgG Removal Reagent contains Sodium Azide as a preservative which may be toxic if ingested. Sodium Azide 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 solution containing Sodium Azide.
4. Remove only the amount of Zorba® IgG Removal Reagent needed to perform each test run to reduce the possibility of product contamination.
5. Zorba® IgG Removal Reagent should be used only as a diluent for patient specimens:
6. Do not use Zorba® IgG Removal Reagent to dilute the Controls or Conjugate.
7. Do not use Zorba® IgG Removal Reagent in any of the wash steps.
8. The volume of Zorba® IgG Removal Reagent 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 insufficient Zorba® IgG Removal Reagent to allow each test well to be utilized.
9. Do not freeze. Specific activity of the reagent may be compromised by freezing and thawing.

MATERIALS PROVIDED

DIL	SPE

Zorba® IgG Removal Reagent**:** Two, 30mL, bottles containing goat anti-human IgG. Ready to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microliter pipettes - 5µL and 50µL.
2. Test tubes - Small volume (recommend 500µL conical microfuge tubes).
3. Timer.
4. Centrifuge (optional).

STORAGE CONDITIONS

2°C – 8°C	Zorba® IgG Removal Reagent

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (6, 7). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If delay in testing is anticipated, store test sera at at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

PROCEDURE

1. Allow Zorba® IgG Removal Reagent to warm to room temperature (20 - 25°C).
2. Dispense 45µL of Zorba® IgG Removal Reagent into an appropriately labeled tube.
3. Add 5µL of patient specimen and mix. The resulting dilution is 1:10.
4. The sample is now ready for testing. No incubation is required. Alternately, if testing is not going to be done immediately, the sample may incubate for ≥ 15 minutes. **NOTE: During this incubation period the Zorba® IgG Removal Reagent may become cloudy as immune complex formation progresses.**

5. Flocculent material will not interfere with the ZEUS IFA Test Systems. The flocculent material may be removed by centrifugation prior to performing the specific IgM test.

LIMITATIONS OF THE ASSAY

Zorba® IgG Removal Reagent was tested to determine the limit of IgG neutralized. When used according this procedure, Zorba® IgG Removal Reagent has been shown to neutralize up to 21mg/mL IgG. Normal adult IgG levels may range from 8 - 16mg/mL (8). Patients with an IgG level exceeding 21mg/mL may require additional treatment to neutralize all IgG.

PERFORMANCE CHARACTERISTICS

Zorba® IgG Removal Reagent was evaluated for the removal of IgG using a turbidimetric methodology. Twenty (20) samples were tested for IgG before and after treatment with Zorba® IgG Removal Reagent. The results of this evaluation are shown in Table 1.

Table 1: Total Serum IgG Concentration by Turbidimetry

Sample	Untreated*	ZORBA Treated*	% Reduction
1	7.3	0.3	95.9%
2	9.1	<0.3	≥96.7%
3	7.8	<0.3	≥ 96.2%
4	7.7	<0.3	≥ 96.1%
5	11.3	0.4	96.5%
6	9.0	0.4	95.5%
7	12.2	0.6	95.1%
8	10.0	0.7	93.0%
9	7.7	0.6	92.2%
10	9.3	<0.3	≥ 96.8%
11	9.0	<0.3	≥96.7%
12	8.4	<0.3	≥96.4%
13	13.6	0.4	97.1%
14	6.5	<0.3	≥95.4%
15	7.6	<0.3	≥96.1%
16	8.4	<0.3	≥96.4%
17	10.4	<0.3	≥97.1%
18	4.7	<0.3	≥94.6%
19	9.8	>0.3	≥96.9%
20	11.3	0.4	96.5%

Efficiency = 95.5% ± 1.3% * mg/mL

The functional removal of IgG was demonstrated by testing 48 samples using ZORBA IgG Removal Reagent and comparing them with a commercially available reagent. There was 100 % agreement between the two IgG removal reagents (See Table #2).

Table 2: IgG Positive

Sample Reactivity	Untreated	ZORBA	Commercial IgG Removal Reagent
HSV-1	17	0	0
HSV-2	17	0	0
CMV	9	0	0
Mycoplasma	5	0	0
Total	48	0	0

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