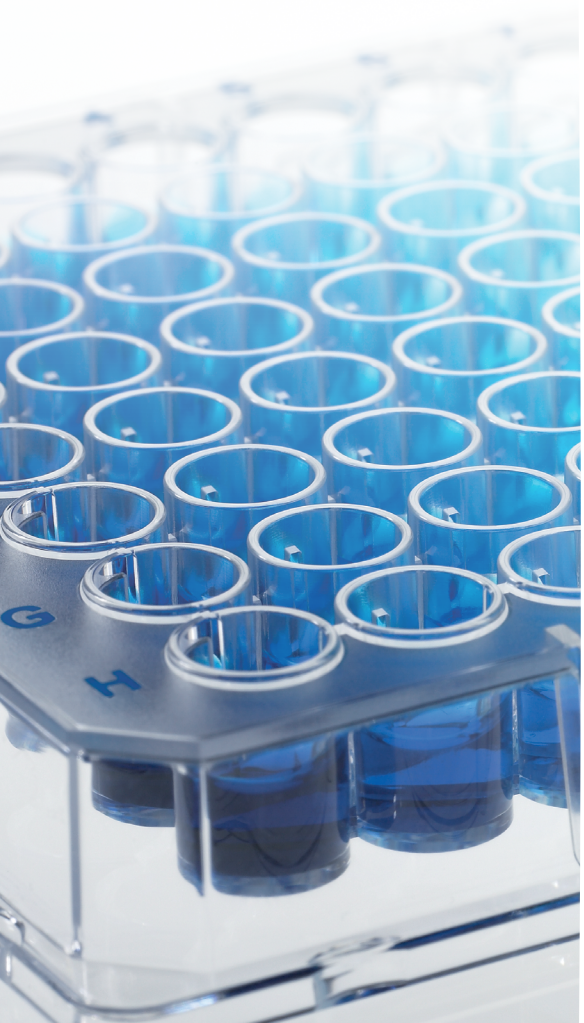
Technical Note

BRAND

Comparison of IgA adsorption capacity between BRAND immunoGrade™ microplates and different competitors

Modern research and development in the field of immunological applications like ELISA, RIA, FIA, etc. is not possible without high-quality plastic disposables.

To ensure reproducible results a consistent quality is needed. In addition to the selection of high quality raw materials with excellent optical characteristics a very homogeneous physical/chemical process leads to a variety of BRAND*plates*® with different binding properties. All BRAND*plates*® microplates can be stored at room temperature and are supplied free from endotoxins, DNA, DNase, RNase and cytotoxic substances. BRAND produces three different surfaces for immunological applications:



1. immunoGrade™:

Basic immunological surface, optimized for the immobilization of IgG and other molecules with hydrophilic and hydrophobic regions.

2. hydroGrade™:

Strongly hydrophilic surface, optimized for hydrophilic molecules like glycoproteins, nucleic acids and proteins with hydrophilic character.

3. lipoGrade™:

Strongly hydrophobic (lipophilic) surface for immobilization of biomolecules with predominantly hydrophobic areas like lipoproteins or peptides.

This is the comparison between BRAND*plates*® immunoGrade™ and some of the high binding surfaces of direct competitors using a direct antibody binding assay.

Materials and methods

1. Chemicals and reagents

– TMB (3,3´, 5,5´-Tetramethylbenzidine, Merck KGaA)

– Hydrogen peroxide 30% (Merck KGaA)

– Tween 20 (Merck KGaA)

– Polyclonal Rabbit AntiHuman IgA-HRP conjugate, ref. P0216 (DAKO North America, Inc.)

– All other reagents were of the highest purity commercially available.

2. 96-well Microplates

– BRAND*plates*® pureGrade™ (non-treated) and immunoGrade™ (BRAND GMBH + CO KG, Germany)

– Competitor A

– Competitor B

– Competitor C

3. Direct antibody adsorption assay

The idea behind the assay is the detection of the binding capacity of the different surfaces using an antibody linked with a peroxidase.

HRP: horse radish peroxidase (enzyme that uses H_2O_2 as substrate)

TMB: (3, 3', 5, 5'-tetramethylbenzidine) is the most commonly used and most sensitive substrate for molecules labelled with the enzyme horse radish peroxidase (HRP). In presence of HRP and H_2O_2 TMB is oxidized to a deep blue product. By addition of acids the product is modified to a yellow molecule with a 2 – 4 times greater molar extinction coefficient than the blue product. Detection is at 450 nm.

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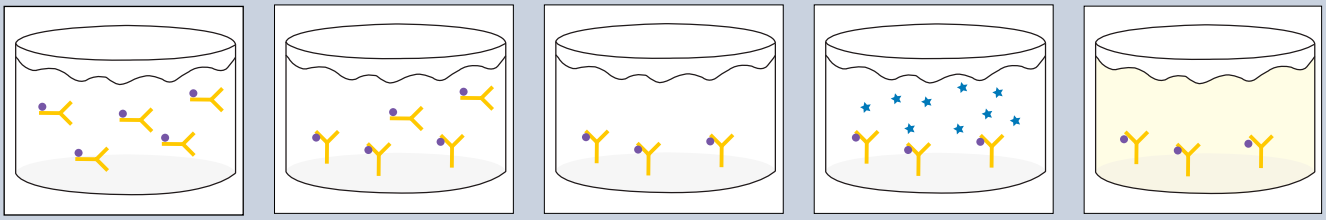


1. Add to a 96-well microplate 100 µl of a rabbit IgA HRP-conjugate with different concentrations (1:4000 to 1:102400) in 100 mM carbonate buffer pH 9.6.

2. Seal plate with an adhesive film and incubate at room temperature for 12 h.

3. Wash 3 times with 0.15 M PBS pH 7.2 containing 0.05% Tween 20.

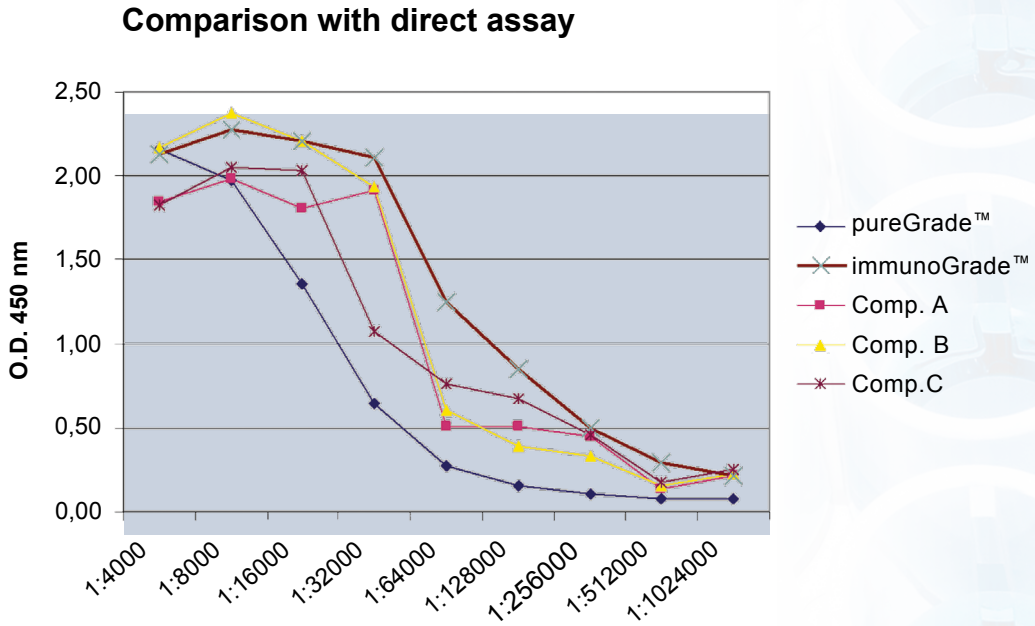
4. Add 100 µl substrate solution (5 % TMB/ 0.04 % H_2O_2).

5. Stop reaction with 150 µl H_2SO_4 and read O.D. at 450 nm using plate reader (SPECTRAmax 384 plus, Molecular Devices, Corp., USA).

antibody HRP-conjugate TMB

Results

Direct antibody adsorption assay



Results of the comparison using a direct IgA adsorption assay

This assay allows the determination of the quantity of protein (IgA) bound on different modified surfaces using the oxidation of TMB. The comparison of BRAND*plates*® microplates immunoGrade™ with untreated PS surfaces and competitors high binding plates shows that the new BRAND*plates*® surface leads to slightly higher adsorption.

Conclusion:

The antibody adsorption on BRAND*plates*® immunoGrade™ surface was compared with high quality high binding surfaces from three competitors.

The new immunoGrade™ surface shows a higher immunoglobulin adsorption compared with competitive products.

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