

# 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 BRANDplates® with different binding properties. All BRANDplates® 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 BRANDplates® 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

- BRANDplates® pureGrade™ (non-treated) and immunoGrade™ (BRAND GMBH + CO KG, Germany)
- Competitor A
- Competitor B
- Competitor C

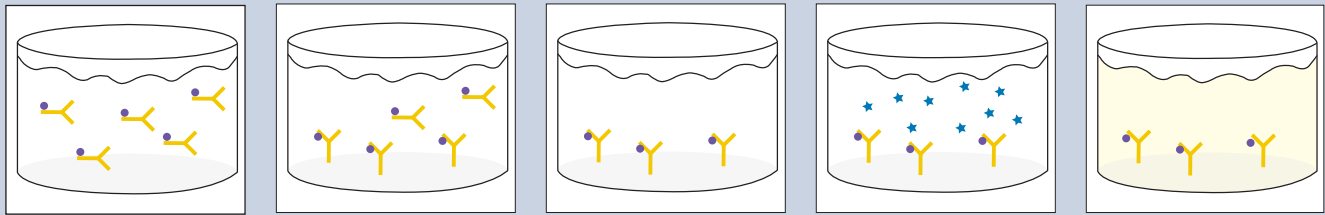
### 3. Direct antibody adsorption assay

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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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.






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<sub>2</sub>O<sub>2</sub>).

5. Stop reaction with 150 µl H<sub>2</sub>SO<sub>4</sub> and read O.D. at 450 nm using plate reader (SPECTRAMax 384 plus, Molecular Devices, Corp., USA).

 antibody

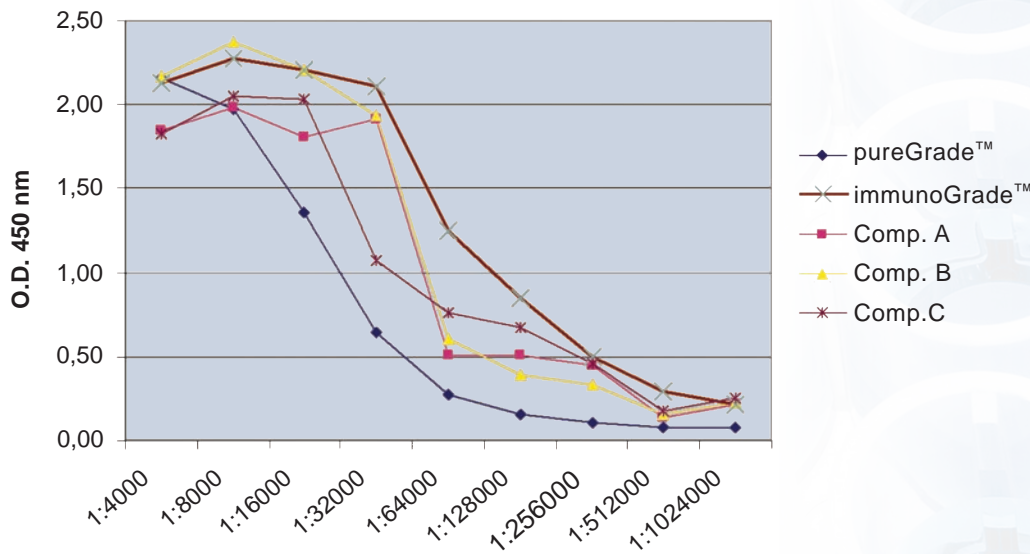
 HRP-conjugate

 TMB

## Results

### Direct antibody adsorption assay

#### Comparison with direct 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 BRANDplates® microplates immunoGrade™ with untreated PS surfaces and competitors high binding plates shows that the new BRANDplates® surface leads to slightly higher adsorption.

## Conclusion:

The antibody adsorption on BRANDplates® 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.**