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 H₂O₂ 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 H₂O₂ 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% H₂O₂).

5. Stop reaction with 150 µl H₂SO₄ and read O.D. at 450 nm using plate reader (SPECTRAmax 384 plus, Molecular Devices, Corp., USA).

**Results**

**Direct antibody adsorption assay**

**Comparison with direct assay**

![Graph showing comparison of antibody adsorption on different surfaces](image)

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.