Supplied by
alpha laboratories
supplying quality to science
Alpha Laboratories Ltd
40 Parham Drive
Hampshire SO50 4NU UK
Tel: +44 23 8048 3000
Email: sales@alphalabs.co.uk
Web: www.alphalabs.co.uk

BRAND

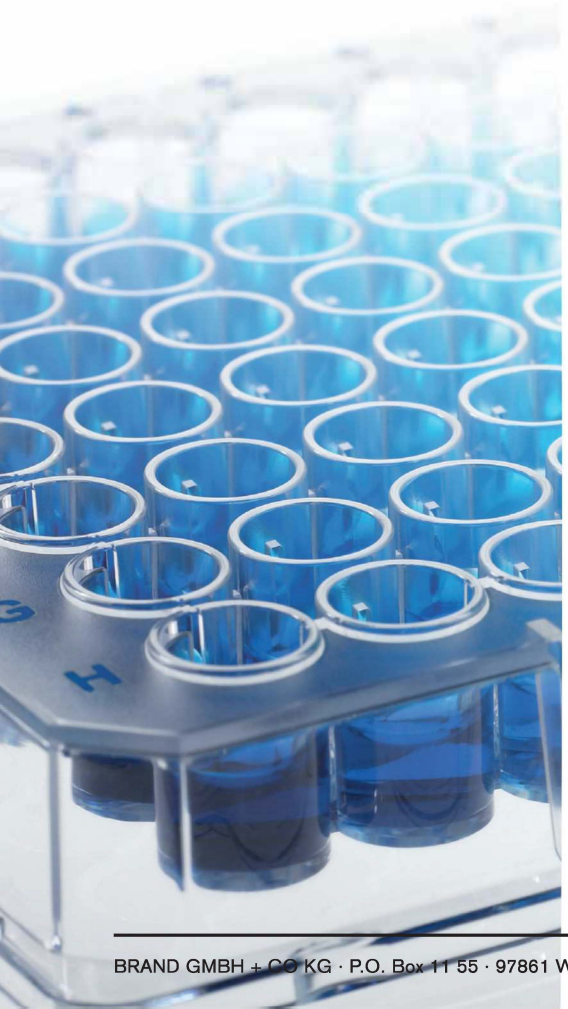
Comparison of IgA adsorption capacity between BRAND immunoGrade™ microplates and different competitors

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.

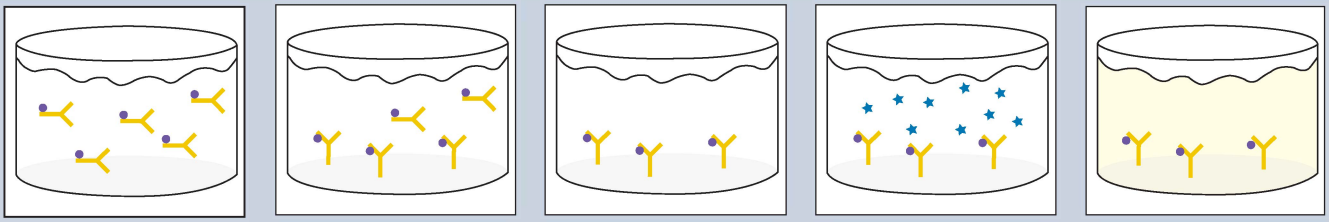
2. 96-well Microplates

- 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.

Supplied by
alpha laboratories
supplying quality to science
Alpha Laboratories Ltd
40 Parham Drive
Hampshire SO50 4NU UK
Tel: +44 23 8048 3000
Email: sales@alphalabs.co.uk
Web: www.alphalabs.co.uk
BRAND



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.

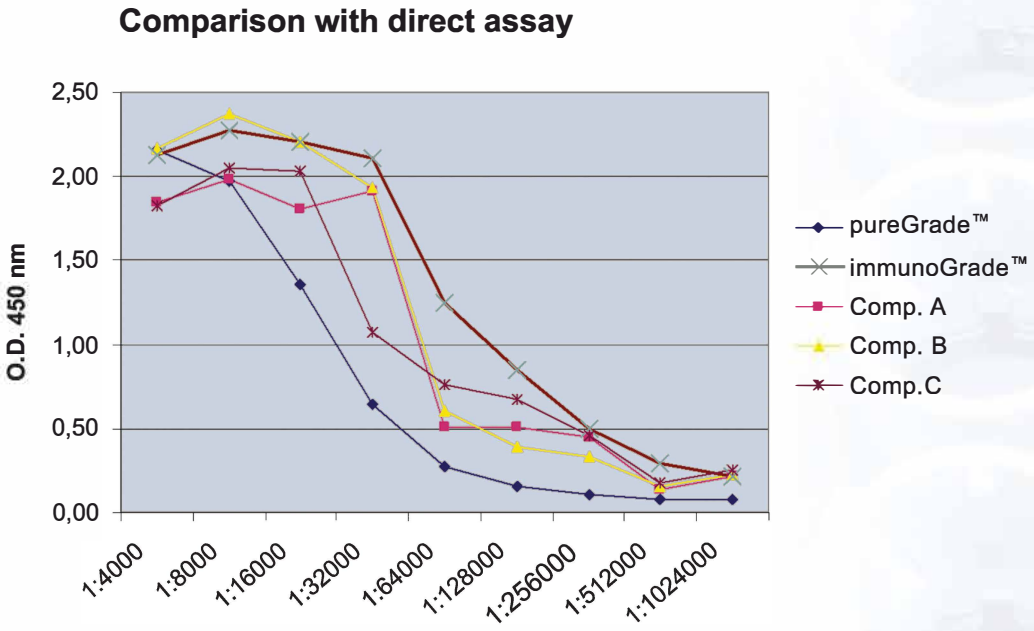
4. Add 100 µl substrate solution (5 % TMB/ 0.04 % H_2O_2).

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.

Printed in Germany · 1114