



## better results in ELISA and Western blotting

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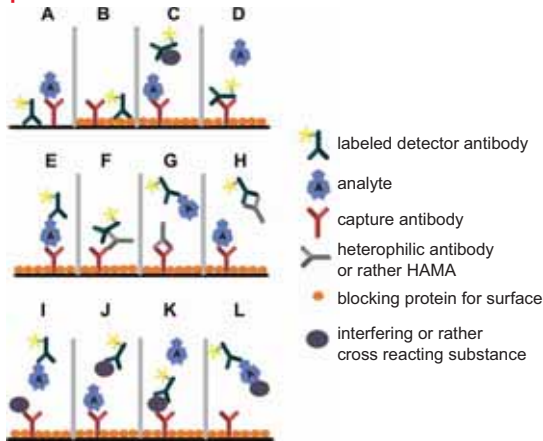
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### Introduction

Interference is a common problem in immunoassays like ELISA, EIA, Western blotting or immunohistochemistry<sup>a,b</sup>. But also modern methods like immuno-PCR, protein arrays and multi-analyte assays suffer from problems like matrix effects, cross-reactivities or unspecific binding<sup>c</sup>. LowCross-Buffer<sup>®</sup> gives simply more reliability and robustness in many assays. Effects of LowCross-Buffer<sup>®</sup> are shown in examples out of the industrial practice.

### Spectrum of interference effects



### Materials and Methods

ELISA against immune globuline from guinea pig, which is used for toxicological studies in guinea pigs. Goat-anti-guinea-pig IgG F(ab')<sub>2</sub> as a capture antibody and goat-anti-guinea-pig IgG (F(c)<sub>γ</sub>) biotinylated as a detector (both Jackson Immunoresearch Laboratories Inc.). The guinea pig IgG was either diluted in LowCross-Buffer<sup>®</sup> (CANDOR Bioscience GmbH) or PBS (range 1-6 50 ng/ml, range 7-12 10 ng/ml). PBS-BSA buffer was used as a blocking buffer. The detection was carried out with Streptavidin-peroxidase (Sigma) and ortho-Phenylendiamine (Sigma). Immunological detection of the cytokeratines 4, 5 and 6 after Western blotting. Lanes 1 and 1' show detection from liver cells and lanes 2 and 2' show detection from HeLa-cells. Blotting membrane is a nitrocellulose-membrane porablot NCP (MACHEREY-NAGEL Co. KG). CRP-ELISA as a model assay for matrix effects done with rabbit serum spiked with a humane C reactive protein (Biotrend). As capture antibody Clone C2 (Biotrend) was used. The spiked serum samples were diluted either with a PBS-BSA buffer or with ImmunoPure<sup>®</sup> TMB-substrate (Pierce).

### Conclusions

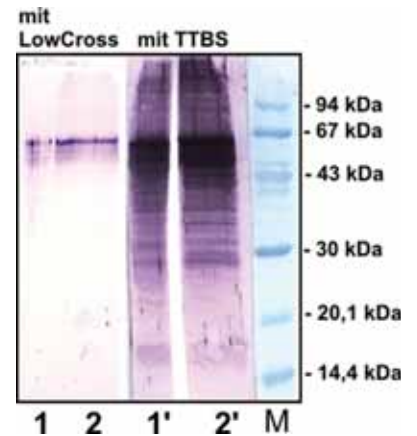
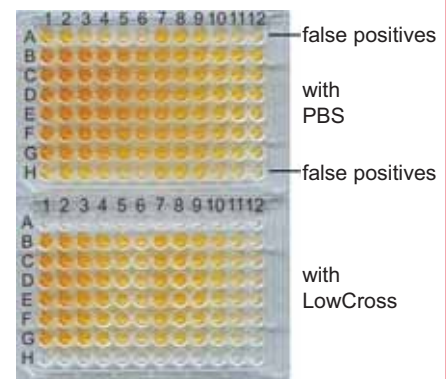
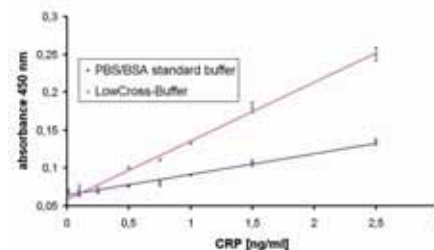
LowCross-Buffer<sup>®</sup> has shown its capability to prevent interference like matrix effects, cross-reactivities or unspecific binding. Interference is a problem leading to unreliable results. In some cases it was just impossible to develop useful assays for routine, because of matrix effects. LowCross-Buffer<sup>®</sup>, which was meanwhile introduced in diagnostics like ELISA-kits and lateral-flow-assays, gives more reliability and robustness to assays. For diagnostical use it is of great importance to avoid false-positives and - much more critical - false-negatives. Therefore LowCross-Buffer<sup>®</sup> gives much more quality and safety to the product and therefore to the diagnostics company.

### Results

Prevention of false-positive binding (control of specificity in row A1-12 and blank row H1-12) by the use of LowCross-Buffer<sup>®</sup> in an ELISA against guinea-pig IgG<sup>d</sup> (Ch. Specht, PARA Bioscience).

Immunological detection of the cytokeratines 4, 5 and 6 after Western blotting<sup>d</sup>. Figure shows the comparison between detection with LowCross-Buffer<sup>®</sup> and TTBS. LowCross-Buffer<sup>®</sup> could prevent unwanted binding totally (D. Sperling, MACHEREY-NAGEL).

ELISA of CRP in rabbit serum. LowCross-Buffer<sup>®</sup> improves the sensitivity by removing a matrix effect<sup>d</sup> (CANDOR Bioscience).



Literature  
[a] Miller, J.J., Clinical Laboratory International 28,2 (2004), 14-17  
[b] Kricka, L.J., Clinical Chemistry 45:7 (1999), 942-956  
[c] Raem, A.M., Rauch, P. (Hrsg) Immunoassays (2006), Elsevier Verlag  
[d] Rauch, P. et al. Laborwelt 4:6 (2005), 33-39