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27E10: A unique monoclonal antibody against MRP8/14 (S100A8/A9, Calprotectin)

Monoclonal antibody 27E10 was developed by generating hybridomas with spleen cells from mice immunized with cultured human monocytes [Zwadlo 1986]. Soon, the description of MRP8 and MRP14 and their association with acute inflammation followed [Odink 1987; Bhardwaj 1992], along with the identification of 27E10 as a Calprotectin-specific monoclonal antibody [Goebeler 1994].

Due to the history of their discovery, MRP8, MRP14, and the heterocomplex MRP8/14 are known under different names. These include MRP8/14 with MRP standing for MIF [Migration Inhibitory Factor] Related Protein, Calprotectin, L1 protein, cystic fibrosis antigen, or S100A8/A9 for the heterocomplex. The 8kDa subunit MRP8 is also known as Calgranulin A, S100A8, or S-100a. The 14kDa subunit MRP14 is also known as Calgranulin B, S100A9, or S-100b.

The naturally occurring MRP8/14 heterocomplex is assumed to consist of three subunits complexed under the influence of Ca^{2+}, two of MRP14 and one of MRP8 [Johne1997]. Calprotectin is unusually resistant against chemical or enzymatic degradation and diagnostically valuable because of the large difference between normal and pathological samples.

In an analytical setting, similar proteins may interact non-specifically and distort the measurement. Partial sequence identity between human MRP8 and MRP14, and with other S100 proteins, need to be addressed and non-specific effects prevented as far as possible. ***Monoclonal antibody 27E10 is an important contribution to improving the specificity of a Calprotectin immunoassay since it reacts very specifically with an epitope which only occurs on the heterocomplex, not on individual subunits***. Together with other highly specific monoclonal antibodies offered by BMA Biomedicals, a robust and reliable immunoassay can be formed.

The following titration curves illustrate the highly specific nature of 27E10 when titrated against recombinant human MRP8 or MRP14, or against natural MRP8/14 (Calprotectin). There is virtually no reaction between 27E10 and either of the recombinant subunits, while the half-maximal reaction with MRP8/14 occurs at about 80ng/ml of 27E10.

BMA Biomedicals owns the license to produce 27E10 and other monoclonals for diagnostic applications and has the capacity to produce gram quantites on request.

Contact us for a quote or for further technical information:

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Figure A: recombinant human MRP8 (Calgranulin A, S100A8) was immobilized on an ELISA plate through a polyclonal antibody, and different monoclonals were titrated and then detected with a secondary HRPO-coupled polyclonal antibody. Clones 8-5C2 and S13.67 are specific for different epitopes on MRP8, 27E10 is specific for MRP8/14.

In this setting, clone 8-5C2 yields a significantly better binding characteristic than S13.67 while 27E10 shows marginal binding even at high antibody concentrations. This may be due to the population of polyclonal antibodies used for capturing MRP8, which looks skewed towards the S13.67 epitope. Figure C (below) indicates that the 8-5C2 epitope may not be exposed on the Calprotectin heterocomplex which may enhance its immunogenicity and hence the high affinity seen in this experiment.

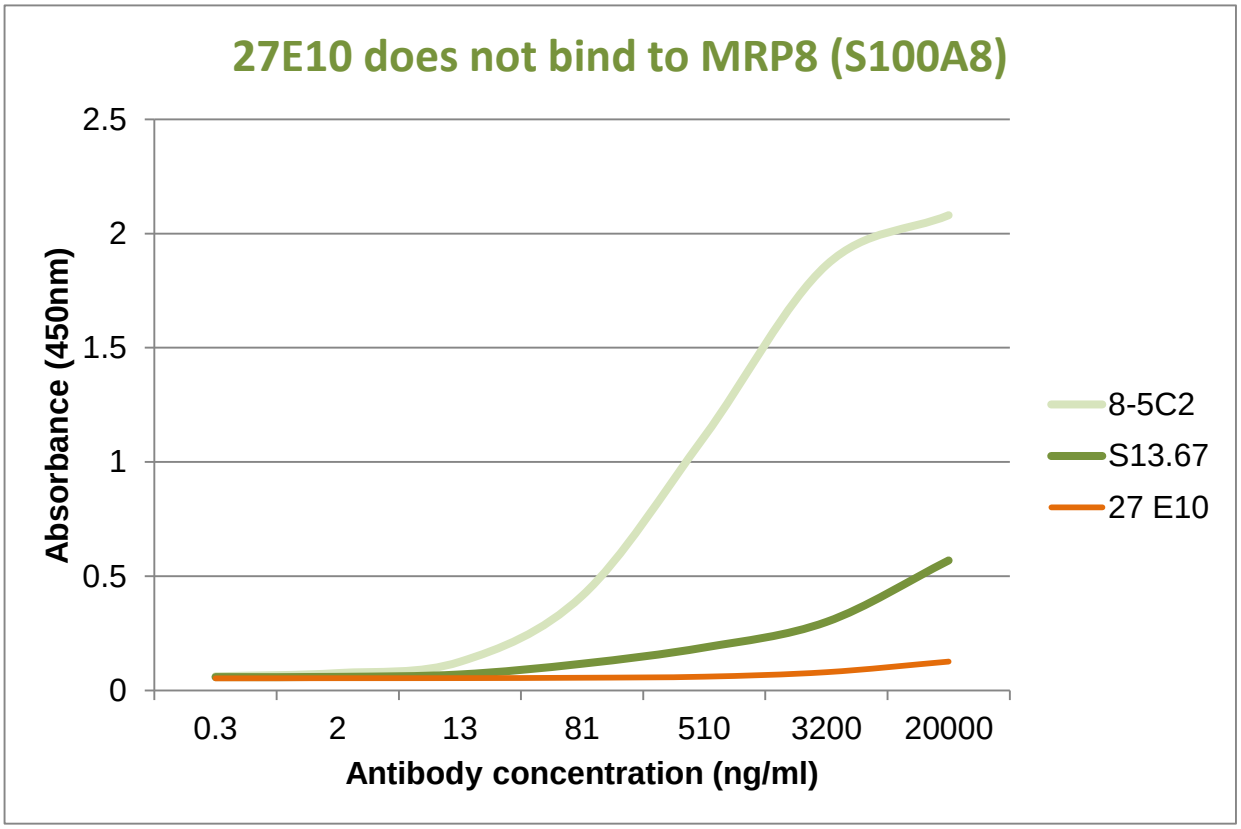


Figure B: recombinant human MRP14 (Calgranulin B, S100A9) was immobilized on an ELISA plate through a polyclonal antibody (same conditions as in figure A), and different monoclonals were titrated and then detected with a secondary HRPO-coupled polyclonal antibody. Clones S32.2 and S36.48 are specific for different epitopes on MRP14, 27E10 is specific for MRP8/14.

In this setting, clone S32.2 yields a significantly better binding characteristic than S36.48 while 27E10 shows marginal binding even at high antibody concentrations. This may be due to the population of polyclonal antibodies used for capturing MRP14, which looks skewed towards the S36.48 epitope. Figure C (below) indicates that the S36.48 epitope is well exposed on the Calprotectin heterocomplex due to its duplication in the Calprotectin trimeric complex which contains two MRP14 subunits.

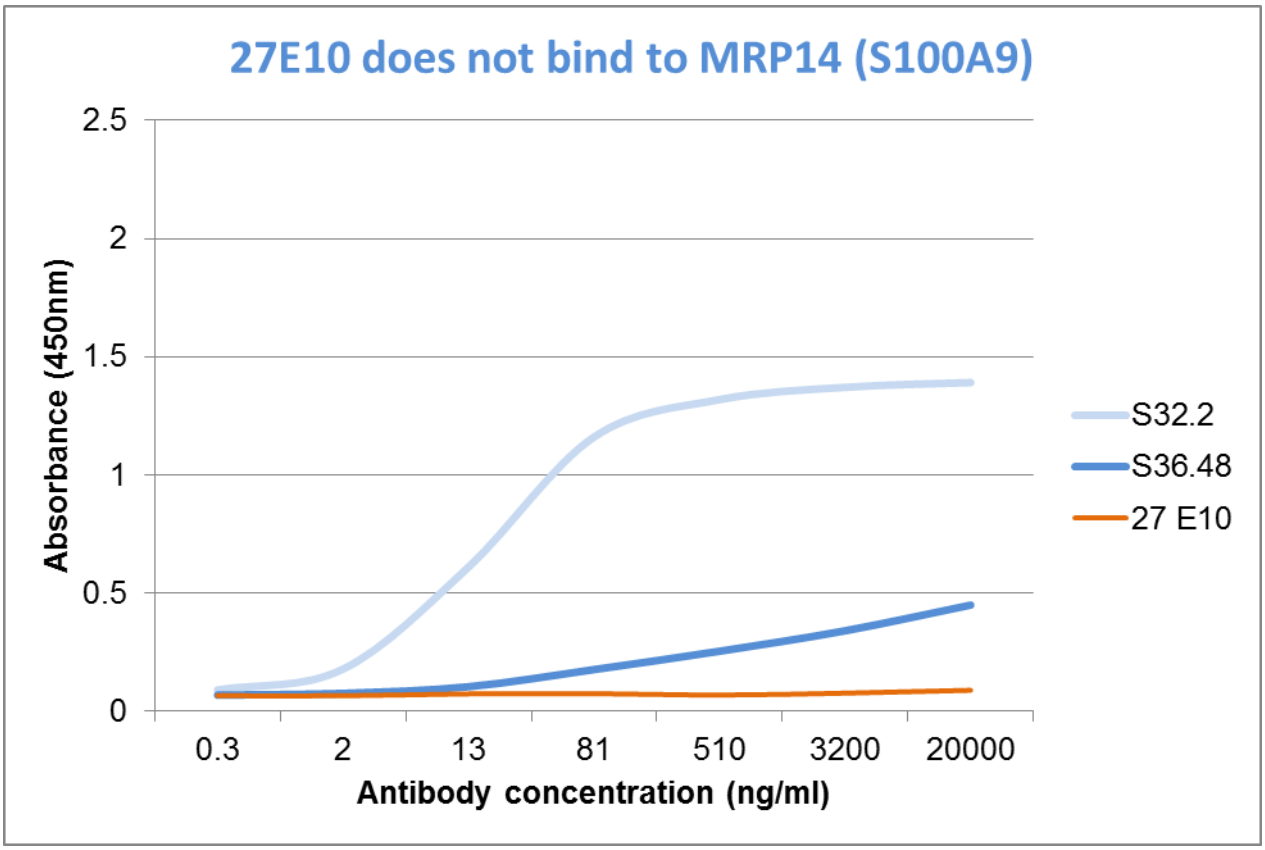
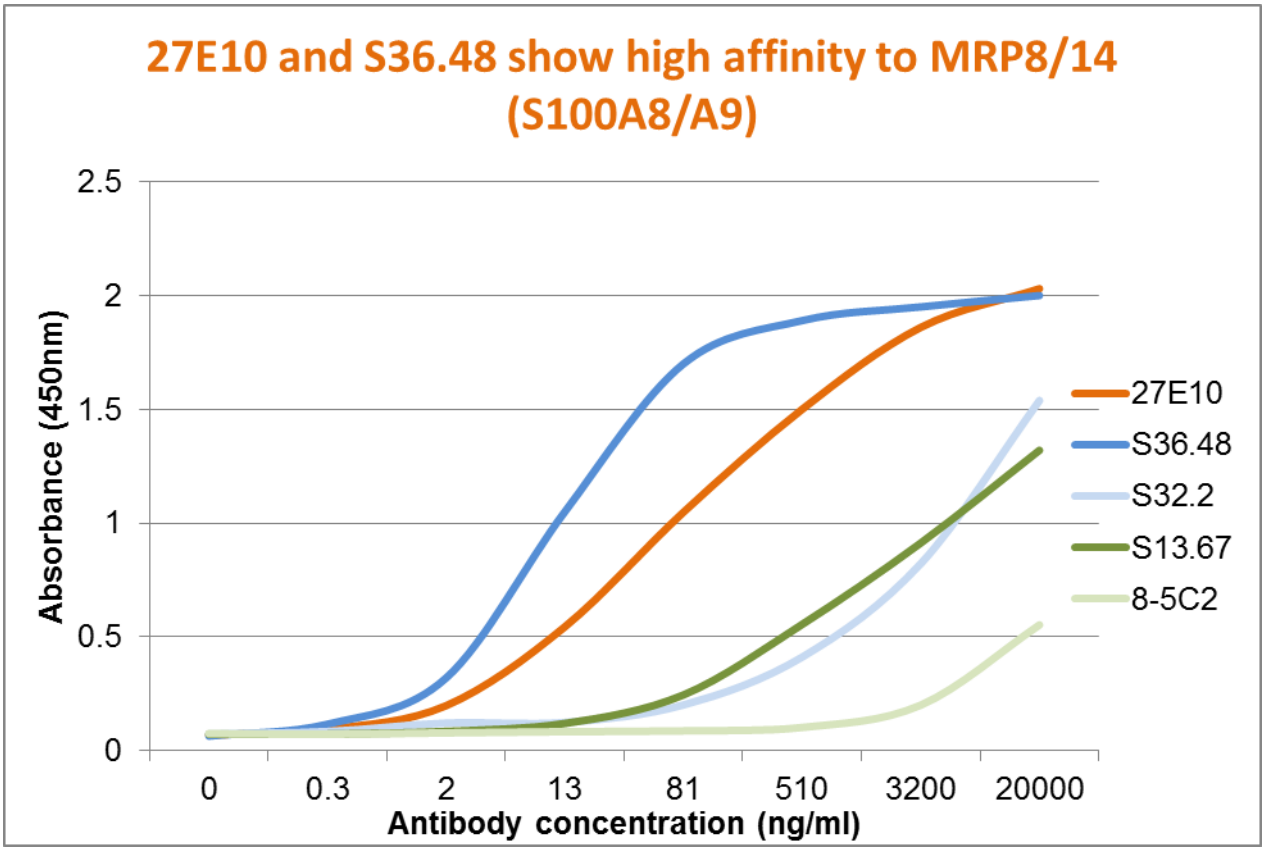


Figure C: natural human MRP8/14 (Calprotectin, 100A8/9) was immobilized on an ELISA plate through a polyclonal antibody (same conditions as in figures A and B), and different monoclonals were titrated and then detected with a secondary HRPO-coupled polyclonal antibody. Clones 8-5C2 and S13.67 are specific for different epitopes on MRP8, S36.48 and S32.2 are specific for two different epitopes on MRP14, and 27E10 is specific for an epitope unique on the MRP8/14 heterocomplex.

In this setting, clone S36.48 shows the highest affinity towards Calprotectin. This is due to the trimeric nature of Calprotectin with a second MRP14 subunit being well exposed for binding by anti MRP14 antibodies. 27E10 shows high affinity as well, contrary to its reactivity with the individual subunits (figures A and B). The anti MRP8 antibodies show lower affinity to MRP8/14 which doesn't come surprisingly when assuming that MRP8 is somehow sandwiched between two MRP14 subunits.

This experiment would favor a Calprotectin ELISA with 27E10 as capture antibody due to its discriminating specificity, and S36.48 as a detection antibody for its high affinity to the Calprotectin heterocomplex.



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