

## PRODUCTION OF AN OLIGOCLONAL ANTIBODY TO ASSAY SPECIFIC CANINE IgE

M GARCÍA-GALLO<sup>†</sup>, L MARTÍN<sup>†</sup>, M LLORENTE<sup>†</sup>, L KREMER<sup>‡</sup>, A MAS<sup>\*</sup>, J ÁLVAREZ<sup>\*</sup>

<sup>\*</sup>ALERGOVET SL, MADRID, Spain

<sup>†</sup>Protein Tools Unit Centro Nacional de Biotecnología/CSIC, MADRID, Spain

<sup>‡</sup>Department of Immunology & Oncology Centro Nacional de Biotecnología/CSIC, MADRID, Spain

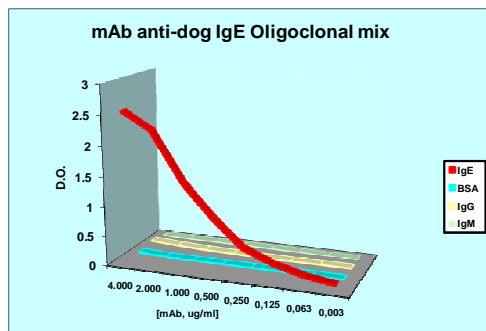
In contrast to polyclonal antibodies, whose use can produce false positive results, the use of highly specific monoclonal antibodies (mAb) often leads to low-sensitivity assays. To address this problem, mixtures of specific mAb can be employed. To date, mAb mixtures generated using recombinant canine IgE (rIgE) as immunogen have not been described. Here we report the generation of a mixture of mAb with high affinity and specificity for canine IgE.

rIgE linked to keyhole limpet hemocyanin (KLH) was injected subcutaneously into mice. Spleen lymphocytes from immunized mice were fused with murine myeloma cells. Culture supernatants were assayed by ELISA and positive hybridomas were cloned. Selected mAb were purified, conjugated to peroxidase and characterized.

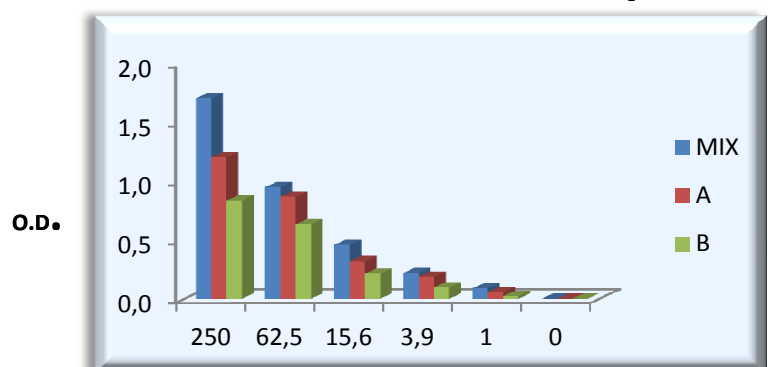
Immunization of mice with rIgE-KLH raised high serum levels of anti-rIgE antibodies. After cell fusion, the supernatants of approximately 18,000 hybridomas were tested in ELISA, 20 of which detected specific canine IgE in sera from atopic dogs. Three selected hybridomas were cloned and mAb from supernatants were purified and conjugated with peroxidase. These mAb recognized canine IgE but not canine IgG/IgM or Bovine Serum Albumin (BSA). Competitive ELISA assays using unconjugated mAb as competitors showed non-overlapping target epitopes for each mAb, allowing their use as part of a mixture or oligoclonal antibody. The mAb mixture showed greater sensitivity for detecting specific canine IgE than each mAb alone.

To summarize, this canine IgE-specific oligoclonal antibody is a novel molecular tool for *in vitro* specific IgE determination in dogs, that increases assay sensitivity while maintaining specificity.

### 100 % IgE Specific



### 30 % more sensitivity



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[IgE, ng/well]