Innovative Rat monoclonal antibody against Hapten

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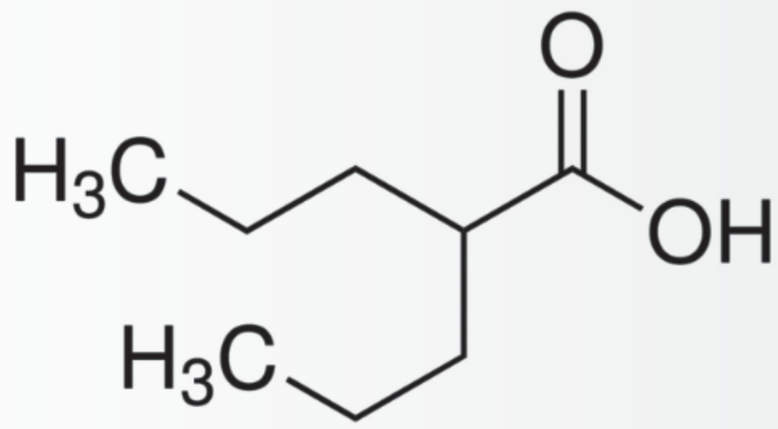
FOREWORD :

Valproic Acid (VP) is a fatty acid with anticonvulsant properties used in the treatment of epilepsy. This small molecule drug is used primarily to control certain seizures by lessening their severity and frequency.

Its level in the blood must be maintained within a narrow therapeutic range, thus the importance of its detection in small quantities. Available mouse monoclonal antibody references don't allow detecting such small quantities and do not have sufficient affinity for the target.

Biokit took the opportunity to collaborate with Synabs given that SynAbs generate mAbs from rats with higher affinity and specificity against small molecules.



PROCEDURE : mAb development

Antigen design

Since VP is a non-immunogenic hapten, it must be previously conjugated to a carrier to trigger a humoral response.

To keep the VP structure, the 6-Amino-2-propylhexanoic acid, a VP analogue (VPA) with a NH2 function was used to form the conjugate with the BSA or KLH carriers.

Immunization

- LOU/C rats (SynAbs proprietary strain) were immunized intraperitoneously (IP) or in footpad (FP) with VPA-BSA or VPA-KLH using a confidential adjuvant (SynAbs proprietary). The footpad response that is specific to the injected antigen permits to avoid the interferences of non-specific natural antigens that may reduce the response against the antigen of interest.

Immunization control

- Immunization control (ELISA) of rats immunized with VPA-BSA were performed on VPA-KLH and vice versa. The best immunized rats were rats immunized with VPA-KLH.

Fusion

- Fusion was carried out with the splenocytes of the best IP immunized rat and with the poplitea lymph nodes lymphocytes of the best FP immunized rat. The fusion was performed by electrofusion with the rat IR983 fusion cell line (Synabs proprietary).

Screening

- Clones supernatants were first screened on VPA-BSA.
- The positive clone supernatants were secondly screened in a competitive ELISA on VPA-BSA in presence of various concentration of free VP. The secondary mAb used for the screening was the mouse anti rat kappa light chain MARk-1 hrp (SynAbs proprietary).

RESULTS :

From 2000 screened clones, 45 clones deplaced by free VP were obtained.

3 clones (27, 30, 44) were selected for their capacity to be deplaced by the lowest free VP concentration. They were isotyped, developed and purified. To purify the rat mAbs, a rat specific Mark-1 column (Synabs proprietary) was used to avoid bovine IgG contaminants.

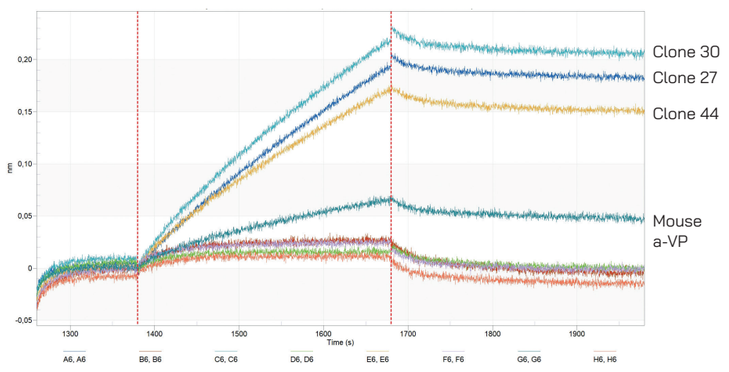
Kinetic and functional assays with these rat mAbs in comparison to the best commercial mouse mAb anti-VP reference were conducted by Biokit :

- **All rat mAbs demonstrate a better affinity compared to mouse mAb**
- **All rat mAbs show a dose-response curve contrary to mouse mAb**
- **SynAbs Clone30 has the highest affinity of the market for free VP (lowest IC50 - 3.5µM).**

Mouse and rat a-VP Kinetic Assay

Kinetic analysis performed using Bio-Layer Interferometry Technology (BLI). Rat/mouse monoclonal antibody coated in carboxylated sensor chip. VP-BSA increasing concentrations: 3.3 to 270nM (Sensorgram curves on the graph corresponds to 3.3nM of VP-BSA).

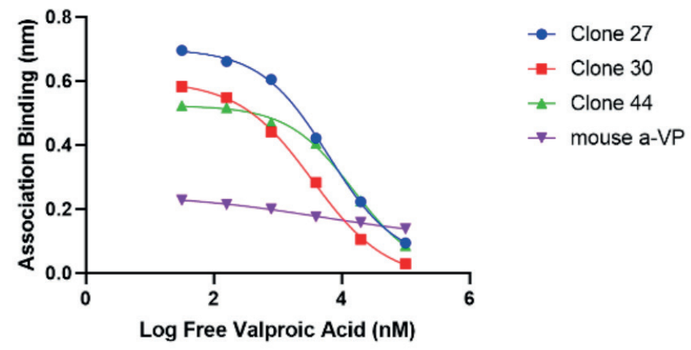
Step selected : Association (Sensor Location, Well Location)



Competitive Binding Assay VP-BSA vs free VP

Competitive binding assay performed using Bio-Layer Interferometry Technology (BLI). Rat/mouse monoclonal antibody-coated in carboxylated sensor chip. VP-BSA fixed concentration + VP free increasing concentrations.

Free Valproic Acid Competition



Commercial Valproic Acid Reagent Kit

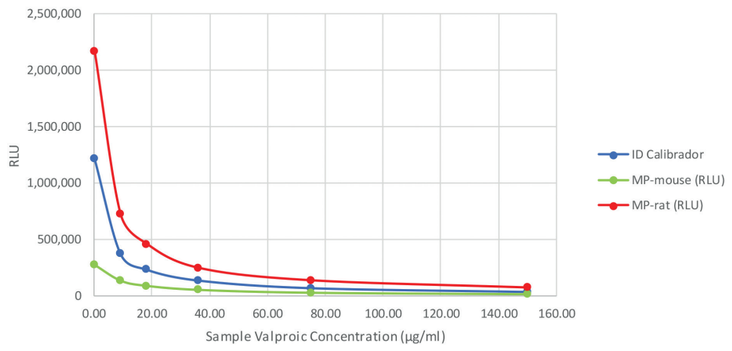
(Chemiluminescent immunoassay)

Sample, anti-valproic acid coated paramagnetic microparticles, and valproic acid-labeled conjugate were combined to create a reaction mixture.

The resulting chemiluminescent reaction is measured as relative light units (RLUs).

An indirect relationship exists between the amount of free valproic acid in the sample and the labeled valproic acid.

Valproic Acid Calibration Curve



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