

DIANA BIOTECHNOLOGIES

DIANA-Ab Case Study:
Development of Anti-GluA1 Recombinant mAb

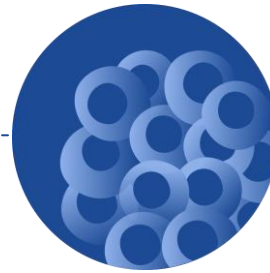
Overview of DIANA-Ab pipeline



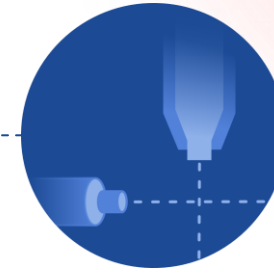
STEP 0.
CUSTOMER
REQUIREMENTS



STEP 1.
IMMUNIZATION



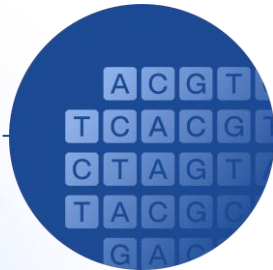
STEP 2.
SPLENOCYTE
ISOLATION



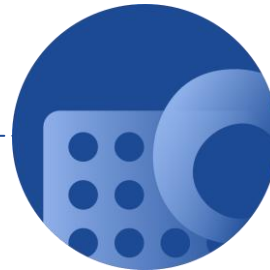
STEP 3.
FACS OF ANTIGEN-
POSITIVE PLASMA
CELLS



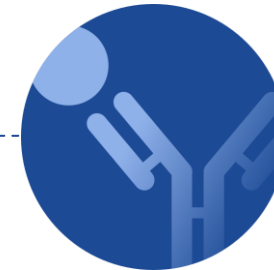
STEP 7.
PURIFICATION &
DIANA-Ab ASSAY



STEP 6.
SEQUENCING
& CLONING

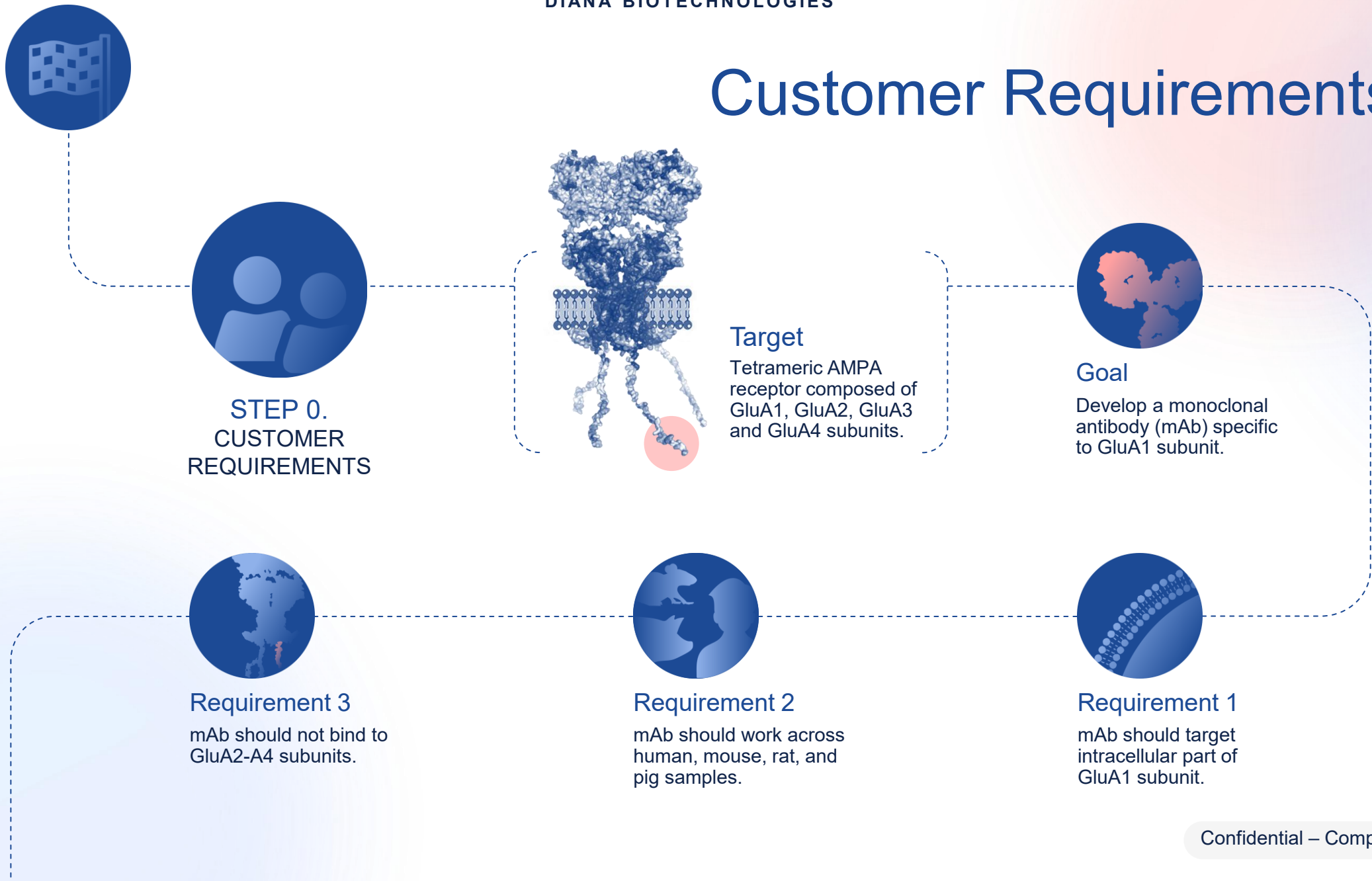


STEP 5.
DIANA-Ab
SINGLE-CELL
SCREENING

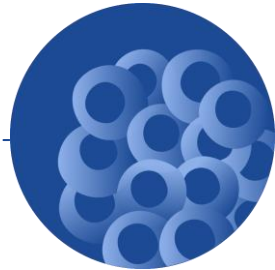


STEP 4.
DIANA-Ab
ANTIGEN-BINDING
CONFIRMATION

Customer Requirements



Splenocytes Isolation & FACS



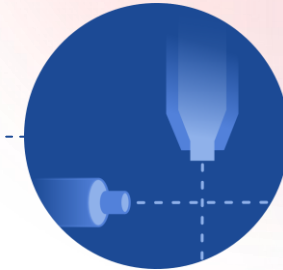
STEP 2.
SPLENOCYTE
ISOLATION

1.96×10^9

splenocytes
isolated in total

1.25×10^8

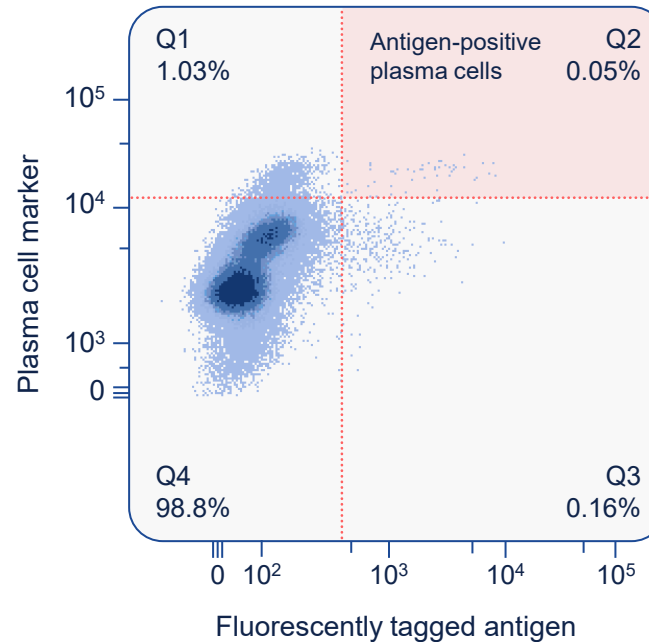
aliquot of splenocytes
used for FACS



STEP 3.
FACS
OF ANTIGEN-
POSITIVE PLASMA
CELLS

7,000

antigen-positive
plasma cells
Identified (Q2)



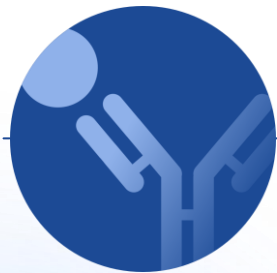
1.40×10^7

live splenocytes
selected

DIANA-Ab Antigen-Binding Confirmation

352

antigen-positive plasma cells were sorted into 384-well plates and tested



STEP 4.
DIANA-Ab
ANTIGEN-BINDING
CONFIRMATION

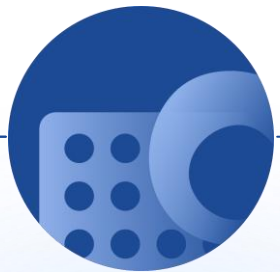
Distribution of 352 clones by their affinity to GluA1 (K_D), pM



212

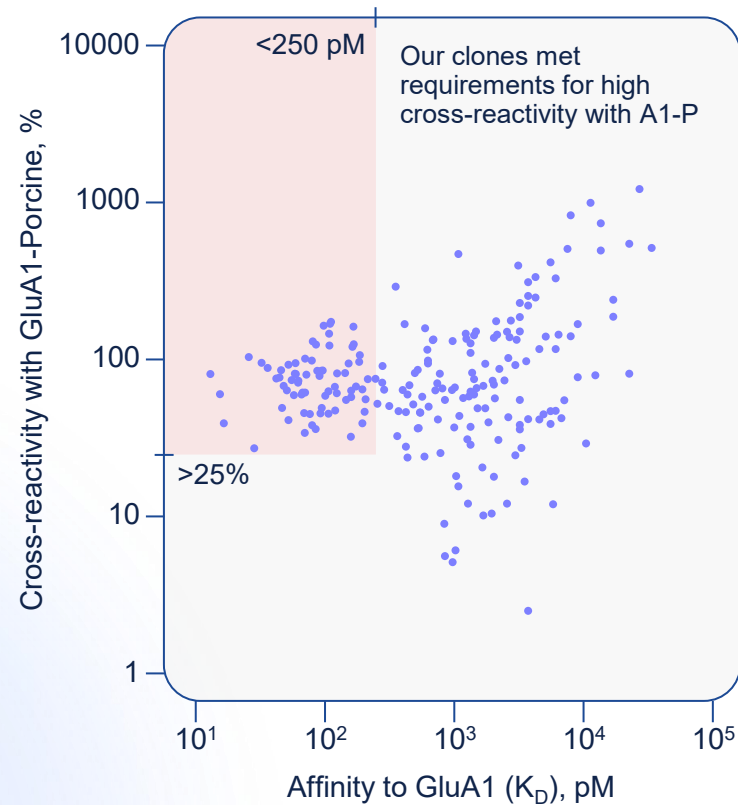
clones with $K_D < 100$ nM to GluA1 were selected for characterization of their affinity and cross-reactivity on a single-cell level

DIANA-Ab Single Cell Screening

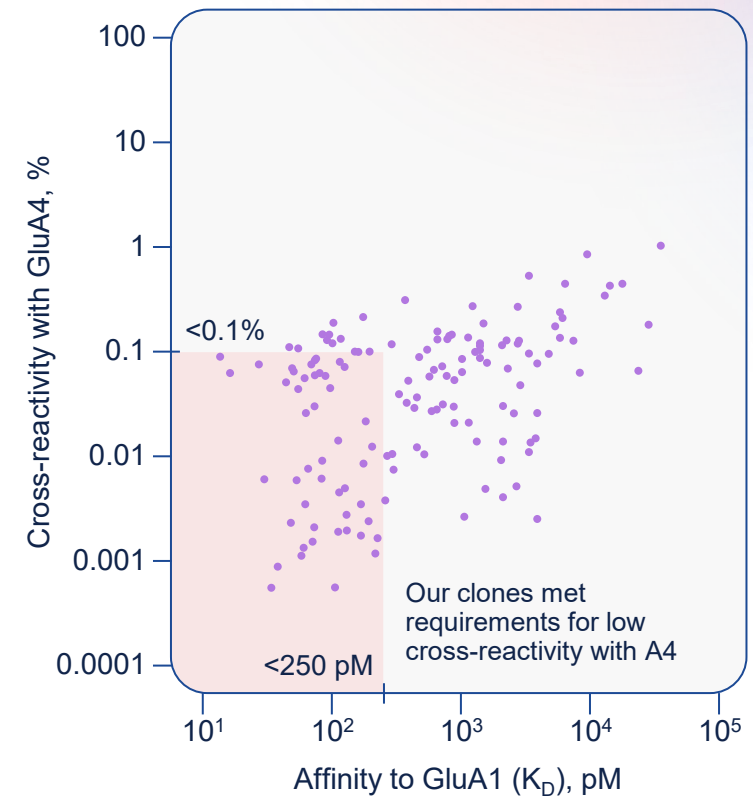


STEP 5. DIANA-Ab SINGLE-CELL SCREENING

Distribution of 212 clones by their affinity to GluA1 (A1)
and cross-reactivity with GluA1-Porcine (A1-P)



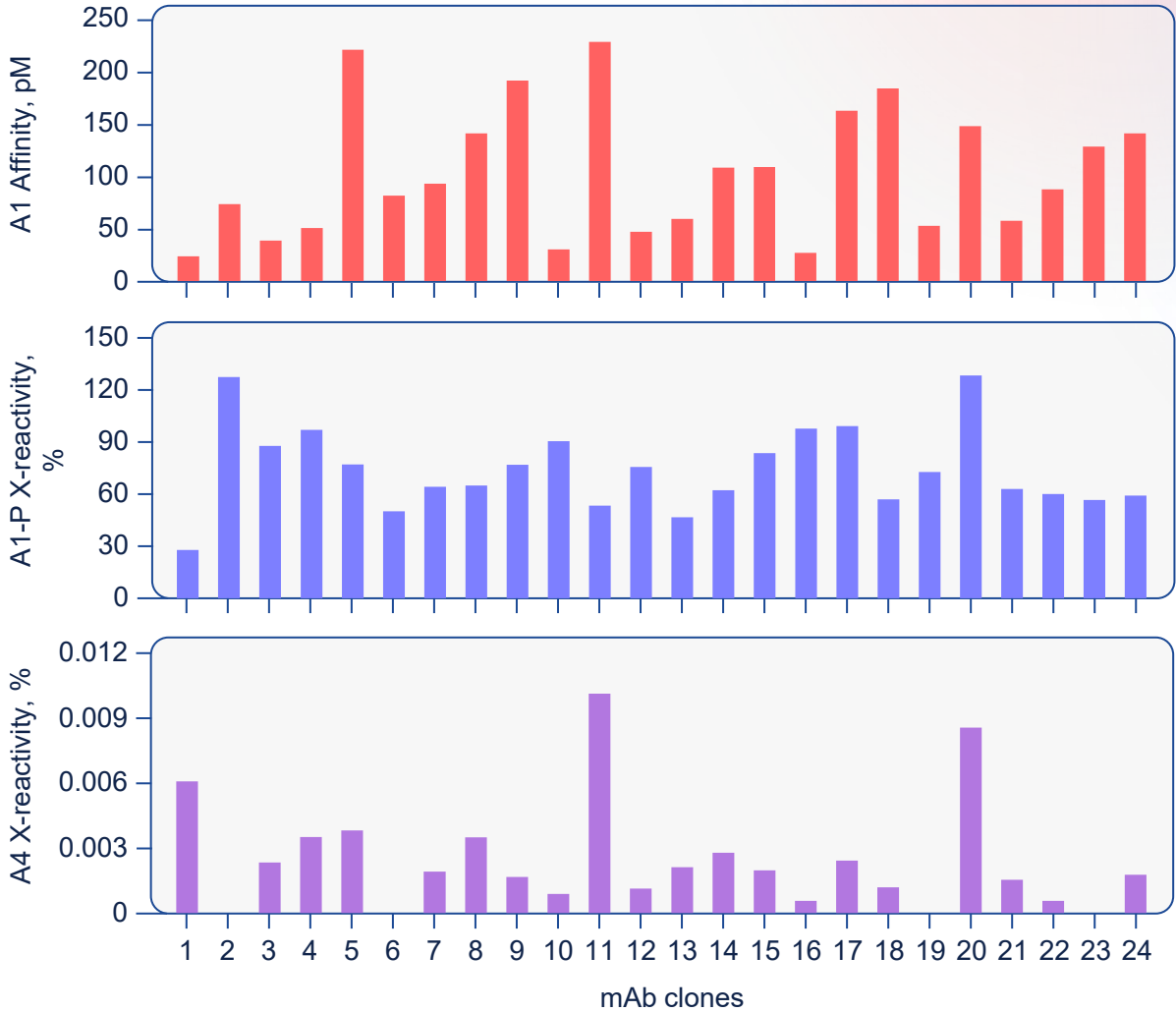
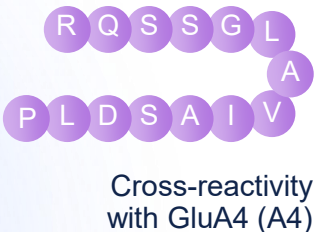
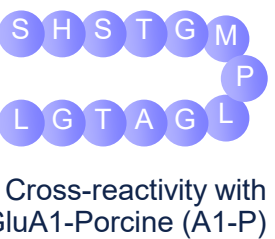
Distribution of 212 clones by their affinity to GluA1 (A1)
and cross-reactivity with GluA4 (A4)



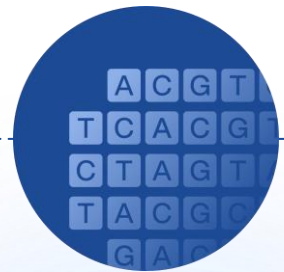
DIANA-Ab Single Cell Screening

24

clones were selected based on their affinities to A1 and their cross-reactivities to A1-P and A4.



Sequencing & Cloning



STEP 6. SEQUENCING & CLONING

Phylogenetic tree of CDR sequences
from 24 clones



96%

sequencing
success rate

Heavy and light chains sequences
were retrieved for 23 out of 24
clones.

22

unique sequences
identified for mAb clones

Phylogenetic tree of CDR sequences
from 24 clones



100%

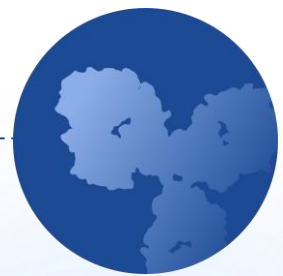
cloning success
rate

10 mAb clones were selected for cloning based on
their affinity, cross-reactivity, and sequence
diversity.

10

clones selected for
cloning & expression

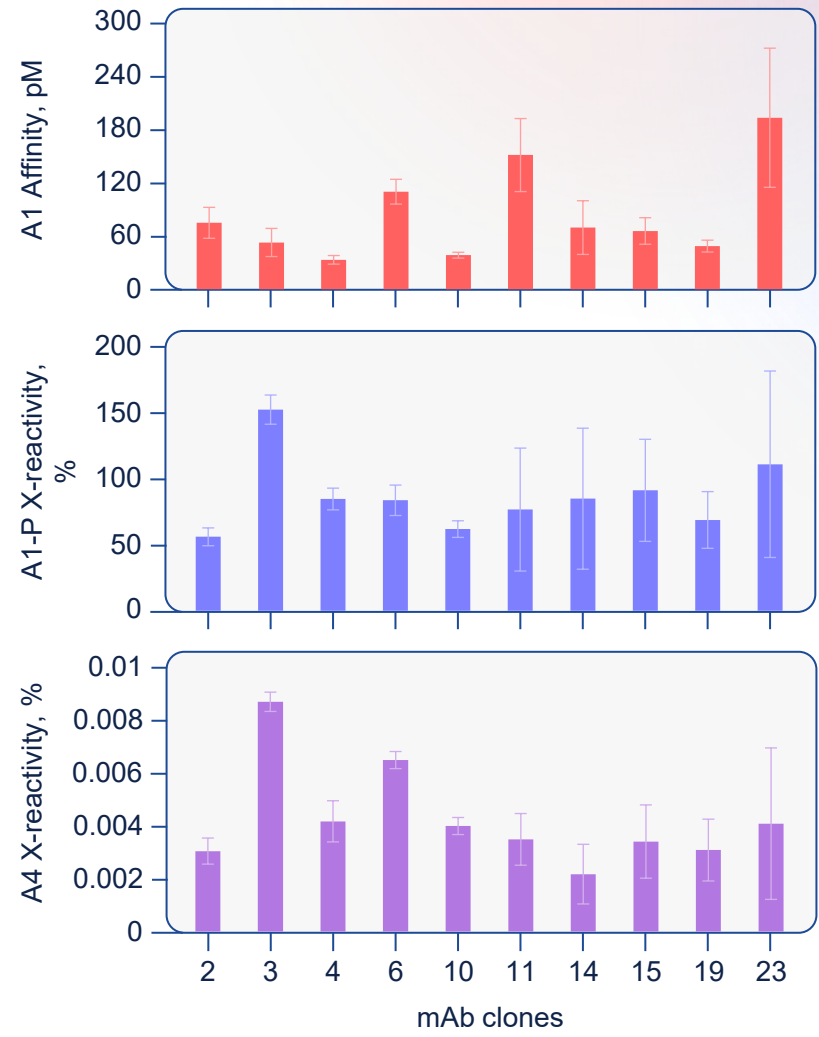
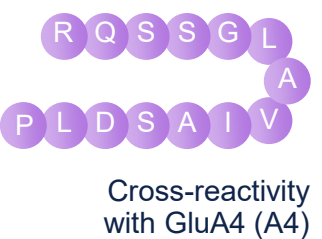
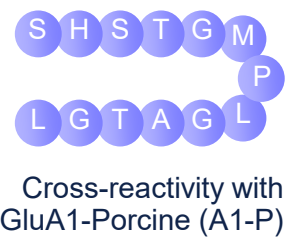
Purification & DIANA-Ab Assay



STEP 7. PURIFICATION & DIANA-Ab ASSAY

10

clones were purified, and their affinities and cross-reactivities were measured using DIANA-Ab assay.



Customer Data

mAb2 clone

showed the best performance in the customer's WB and IP experiments and was selected for licensing

Immunodetection (WB) and immunoprecipitation (IP) of GluA1 subunit using our mAb2 antibody

1

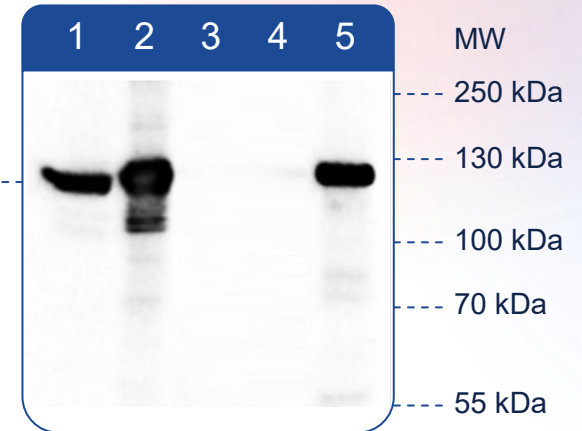
Immunodetection of recombinant GluA1 subunit from HEK293 cell lysate;

2

Immunoprecipitation of recombinant GluA1 subunit from HEK293 cell lysate using mAb2 antibody. Detection by anti-GluA1 rabbit polyclonal antibody AB31232 (Abcam);

4

Immunoprecipitation of recombinant GluA3 subunit from HEK293 cell lysate using mAb2 antibody. Detection by anti-GluA1 rabbit polyclonal antibody AB31232 (Abcam);



3

Immunoprecipitation of recombinant GluA2 subunit from HEK293 cell lysate using mAb2 antibody. Detection by anti-GluA1 rabbit polyclonal antibody AB31232 (Abcam);

5

Immunoprecipitation of GluA1 subunit from pig brain using mAb2 antibody. Detection by anti-GluA1 rabbit polyclonal antibody AB31232 (Abcam).