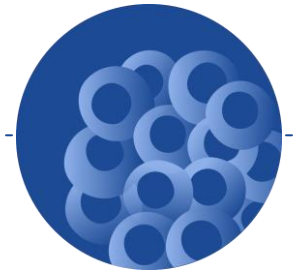
DIANA BIOTECHNOLOGIES

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

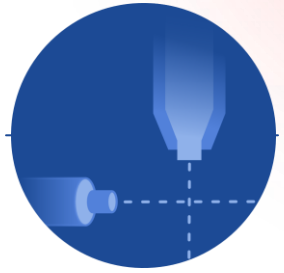
Overview of DIANA-Ab pipeline

STEP 0.
CUSTOMER REQUIREMENTS

STEP 1.
IMMUNIZATION



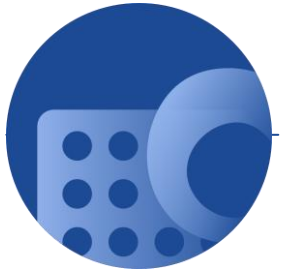
STEP 2.
SPLENOCYTE ISOLATION



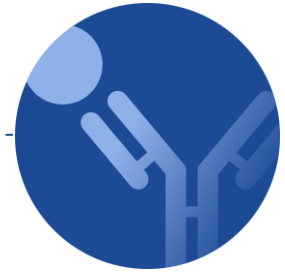
STEP 3.
FACS OF ANTIGEN-POSITIVE PLASMA CELLS

STEP 7.
PURIFICATION & DIANA-Ab ASSAY

STEP 6.
SEQUENCING & CLONING



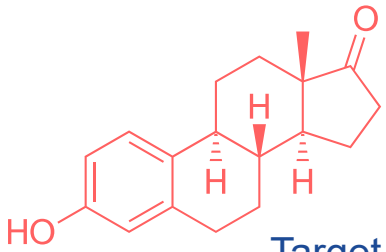
STEP 5.
DIANA-Ab SINGLE-CELL SCREENING



STEP 4.
DIANA-Ab ANTIGEN-BINDING CONFIRMATION

Customer Requirements

STEP 0.
CUSTOMER REQUIREMENTS



Target
Estrone, steroid hormone

Goal
Develop a monoclonal antibody (mAb) specific to Estrone (E1).

Requirement 1
mAb should have <100 pM affinity to estrone (E1).

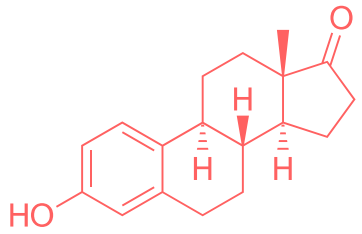
Requirement 2
mAb should have <1.5 % cross-reactivity with Estrone-sulfate (E1-S).

Requirement 3
mAb should have <1.5 % cross-reactivity with Estradiol (E2).

Immunization

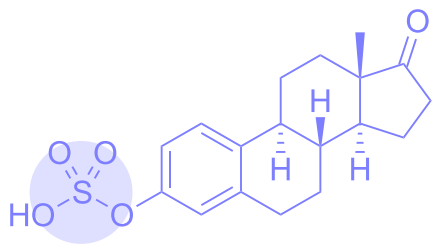
Immunogen and cross-reactants design

Estrone is a small steroid hormone with inherently low immunogenicity. Using literature, hapten design principles, and our advanced expertise in small-molecule and conjugation chemistry, we designed an estrone immunogen. In addition, two structurally related estrogen analogs were selected as cross-reactants.



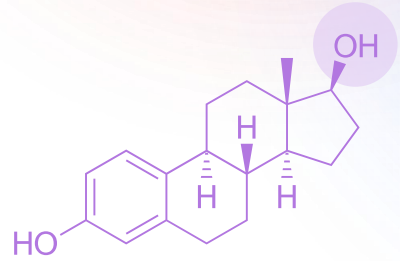
Estrone (E1)
Hapten immunogen

Natural steroid estrogen that becomes the predominant circulating form after menopause.



Estrone-sulfate (E1-S)
Negative cross-reactant

Circulating storage form of estrogen that serves as a major reservoir for estrone and estradiol in peripheral tissues.



Estradiol (E2)
Negative cross-reactant

The most potent natural steroid estrogen and the dominant circulating form during the reproductive years.

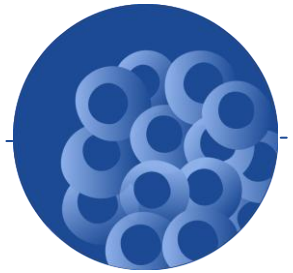
STEP 1.
IMMUNIZATION

Immunization time
9 weeks

Host species
Rabbit

Immunogen
Estrone-VLP conjugate

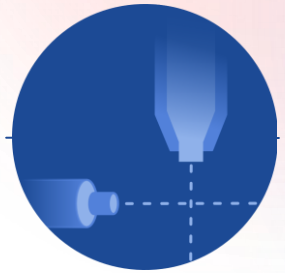
Splenocytes Isolation & FACS



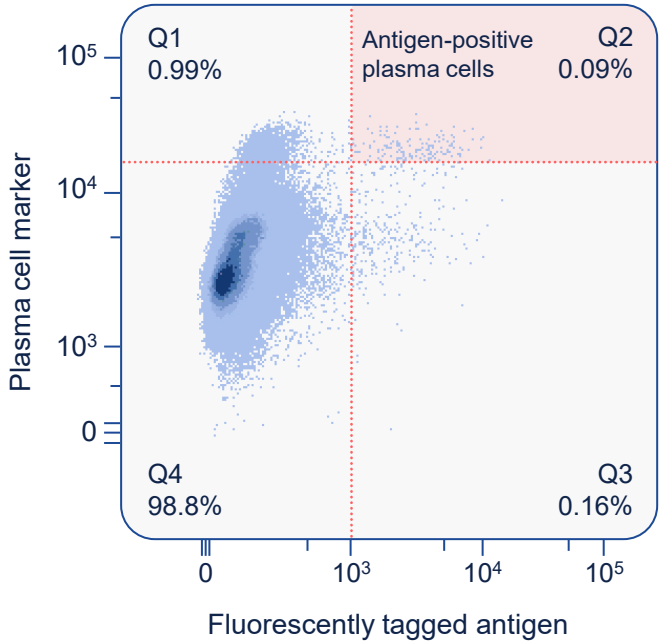
STEP 2.
SPLENOCYTE
ISOLATION

1.16×10^9
splenocytes
isolated in total

1.25×10^8
aliquot of splenocytes
used for FACS



STEP 3.
FACS
OF ANTIGEN-
POSITIVE PLASMA
CELLS



21,000
antigen-positive
plasma cells
Identified (Q2)

2.34×10^7
live splenocytes
selected

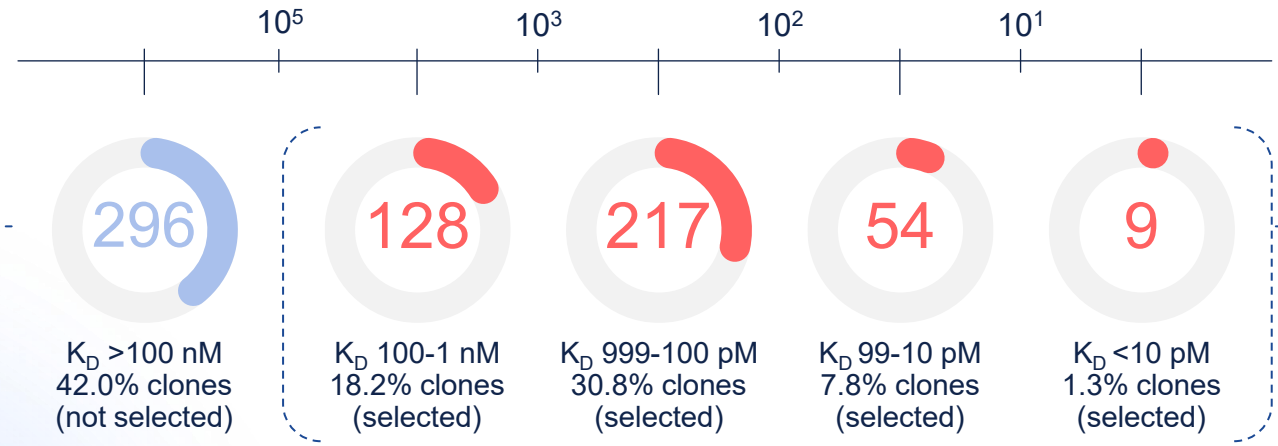
DIANA-Ab Antigen-Binding Confirmation

704

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

STEP 4.
DIANA-Ab ANTIGEN-BINDING CONFIRMATION

Distribution of 704 clones by their affinity to estrone (K_D), pM



K_D >100 nM	K_D 100-1 nM	K_D 999-100 pM	K_D 99-10 pM	K_D <10 pM
42.0% clones	18.2% clones	30.8% clones	7.8% clones	1.3% clones
(not selected)	(selected)	(selected)	(selected)	(selected)

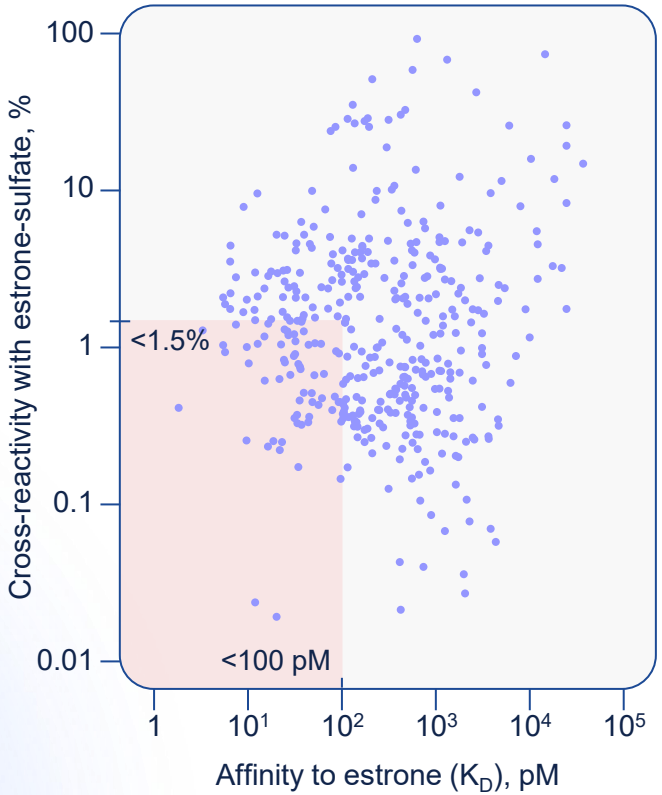
408

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

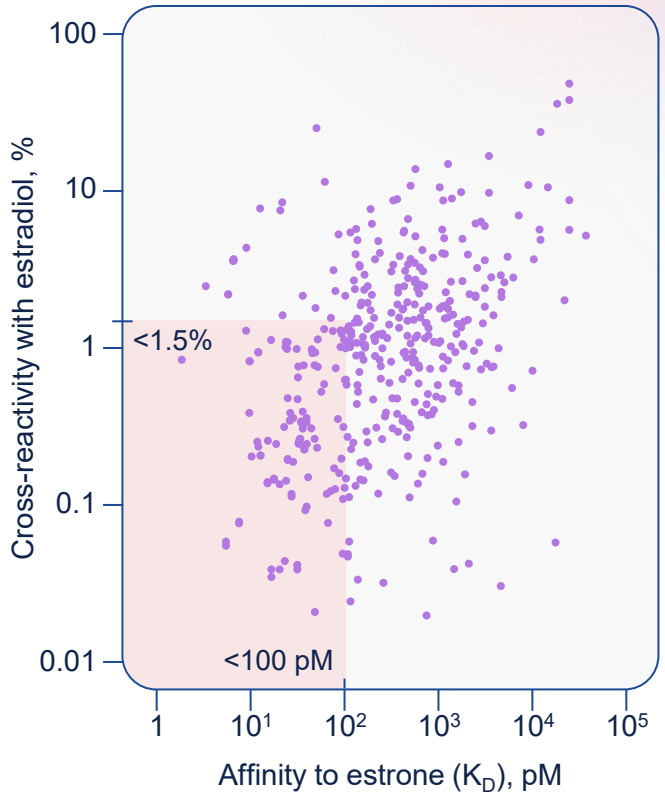
DIANA-Ab Single Cell Screening

STEP 5.
DIANA-Ab
SINGLE-CELL
SCREENING

Distribution of 408 clones by their affinity to estrone (E1) and cross-reactivity with estrone-sulfate (E1-S)



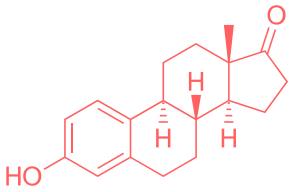
Distribution of 408 clones by their affinity to estrone (E1) and cross-reactivity with estradiol (E2)



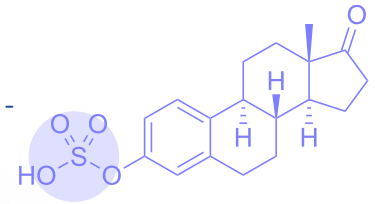
DIANA-Ab Single Cell Screening

48

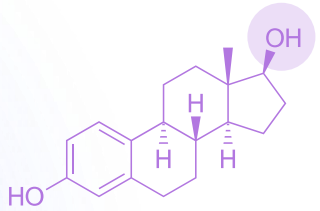
clones were selected based on their affinities to E1 and their cross-reactivities to E1-S and E2.



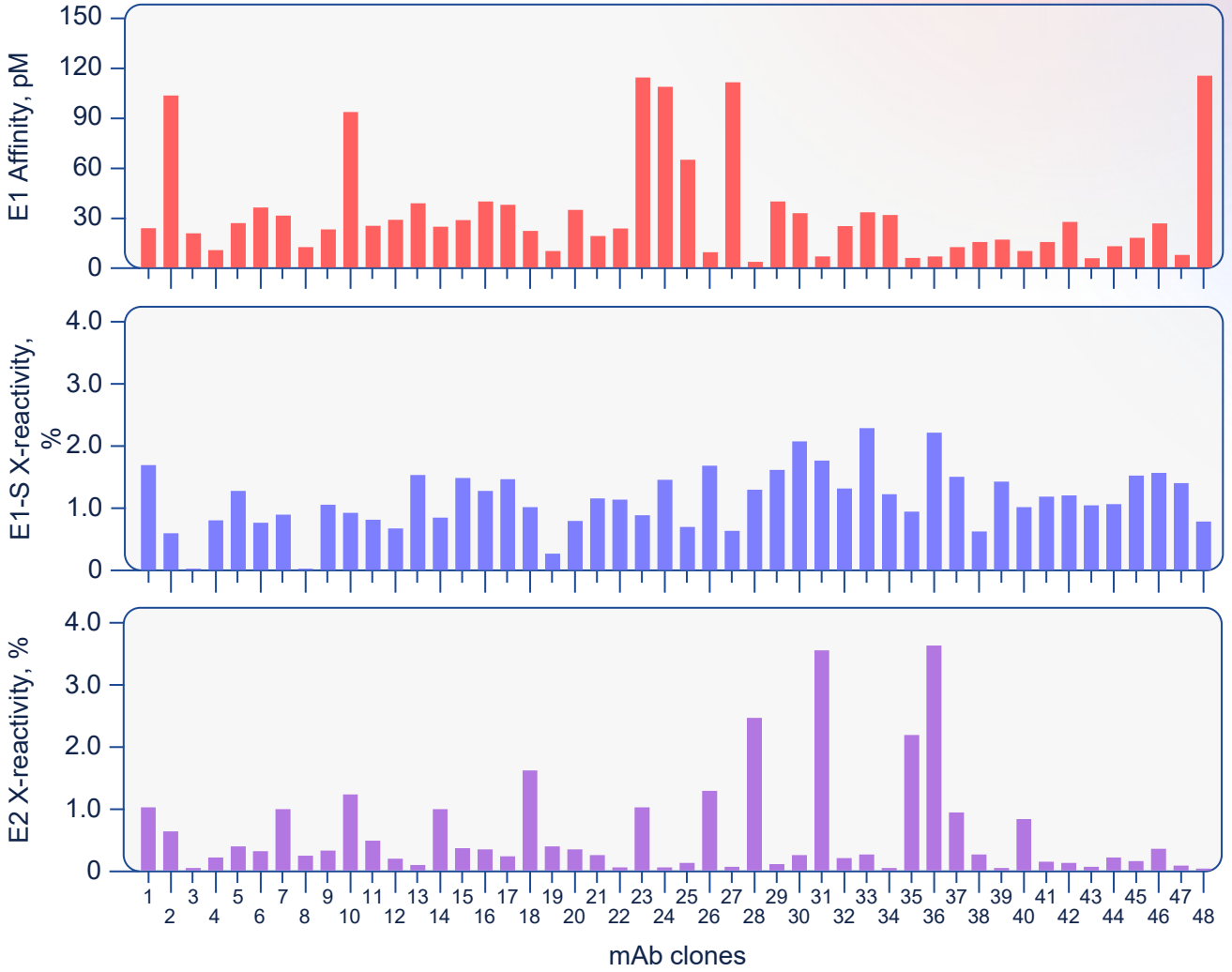
Affinity to Estrone (E1)



Cross-reactivity with Estrone-sulfate (E1-S)



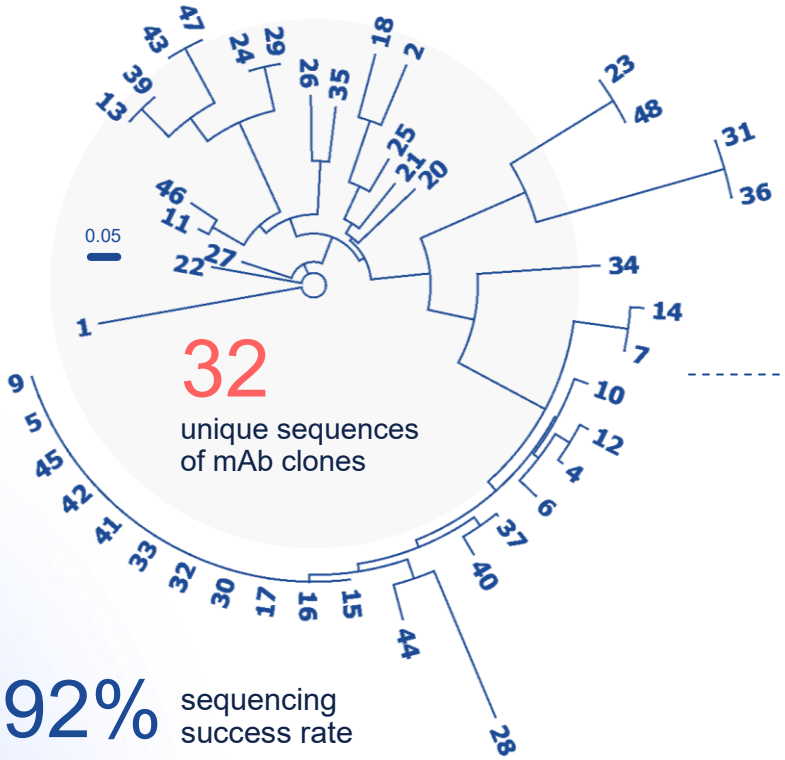
Cross-reactivity with Estradiol (E2)



Sequencing & Cloning

STEP 6.
SEQUENCING
& CLONING

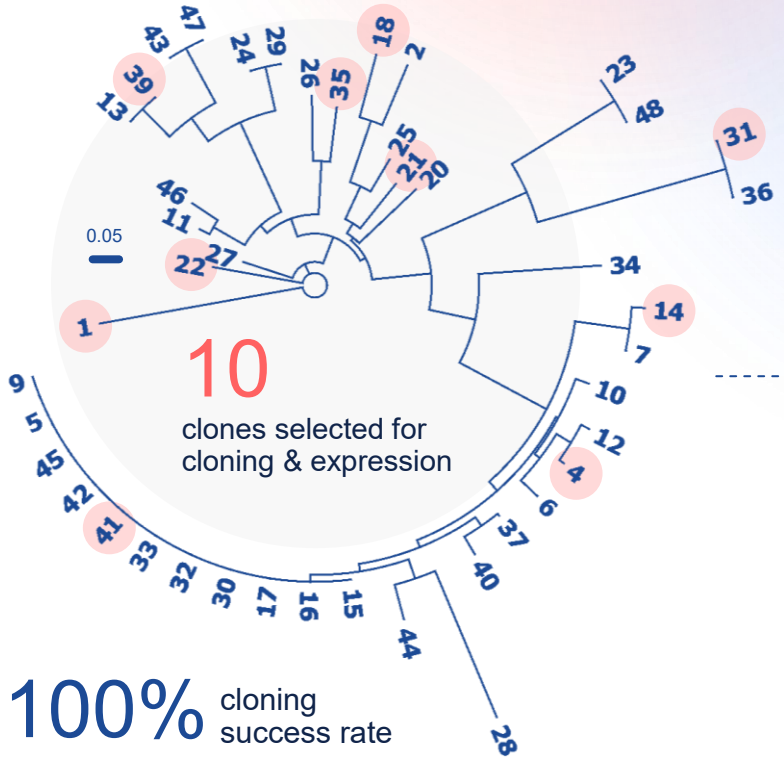
Phylogenetic tree of CDR sequences
from 48 clones



92% sequencing success rate

Heavy and light chains sequences were retrieved for 44 out of 48 clones.

Phylogenetic tree of CDR sequences
from 48 clones



100% cloning success rate

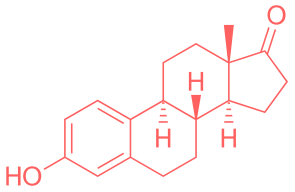
10 mAb clones were selected for cloning based on their affinity, cross-reactivity, and sequence diversity.

Purification & DIANA-Ab Assay

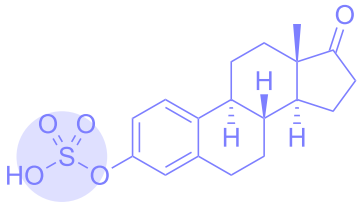
STEP 7.
PURIFICATION &
DIANA-Ab ASSAY

10

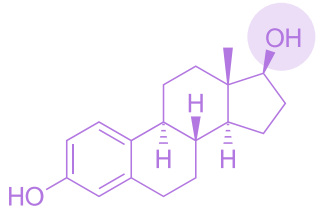
clones were purified, and their affinities and cross-reactivities were measured using DIANA-Ab assay.



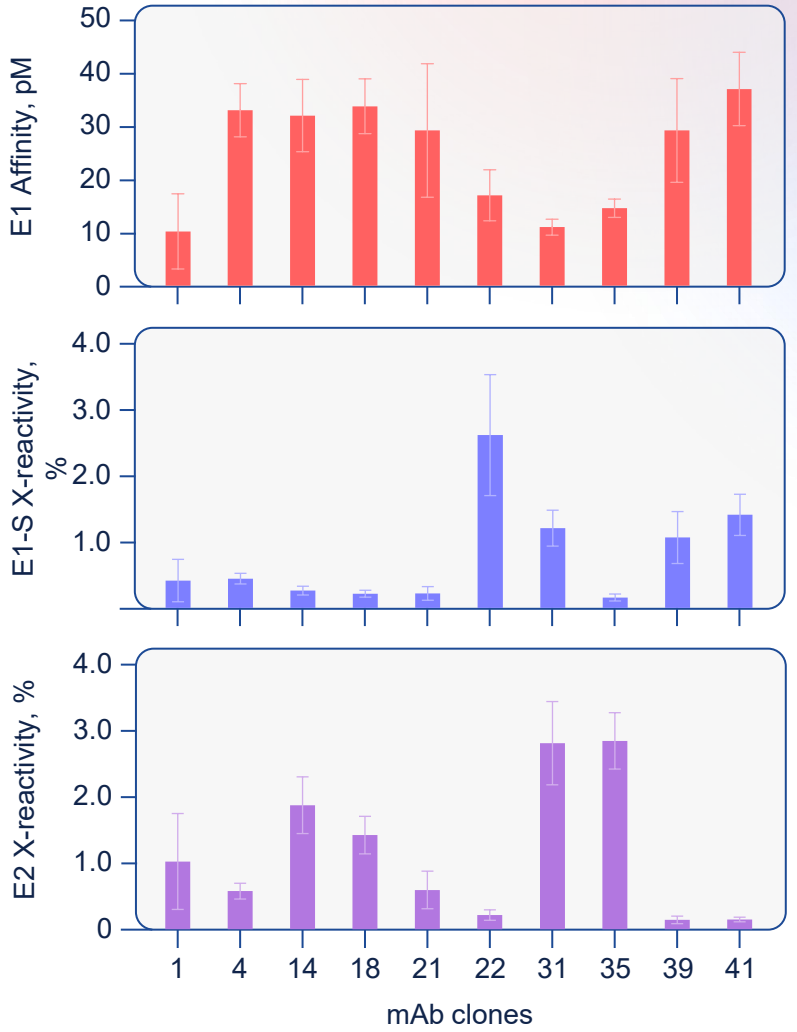
Affinity to
Estrone (E1)



Cross-reactivity with
Estrone-sulfate (E1-S)



Cross-reactivity
with Estradiol (E2)



Customer Clone Selection

mAb21 clone

showed the best performance in the customer's CLIA/RIA assay and was selected for licensing.