



Giulio Russo

Recombinant mono- and multi-clonal antibodies as higher quality substitutes for animal sera in diagnostics

15.10.2020 | Antibody Webinar Series - PETA International Science Consortium

Phage display mAbs in therapy

- 9 animal free phage display-derived therapeutic mAbs approved
- 53 animal free phage display-derived therapeutic mAbs in clinical trials (Aug. 2020)



(Atezolizumab, Avelumab, Belimumab, Necitumumab, Ramucirumab, Guselkumab, Lanadelumab, Emapalumab, Raxibacumab)

Shim H. Curr Pharm Des. 2016, 22:6538-59 | Alfaleh et al., 2020, doi: 10.3389/fimmu.2020.01986

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Rec Abs advantages for therapy

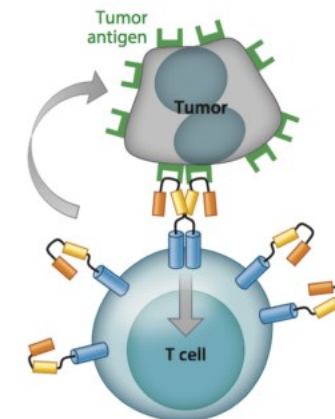
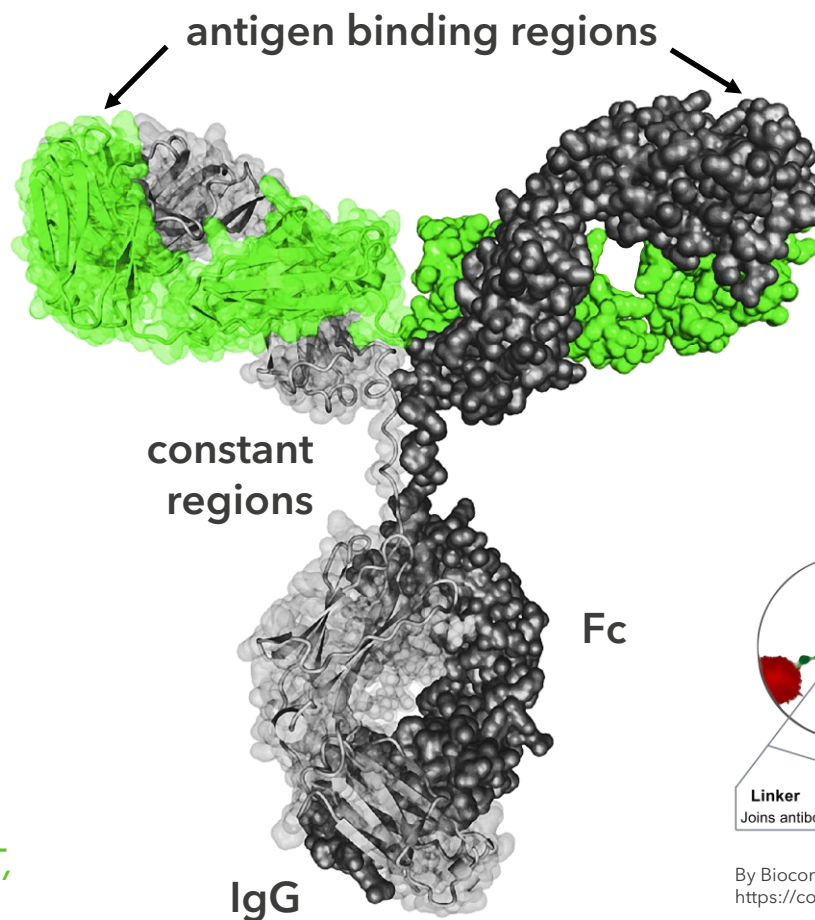
animal free generation
from human naïve libraries
fully human IgG antibodies

sequence defined reagent
*unlimited reproducibility
and scalable batch size*

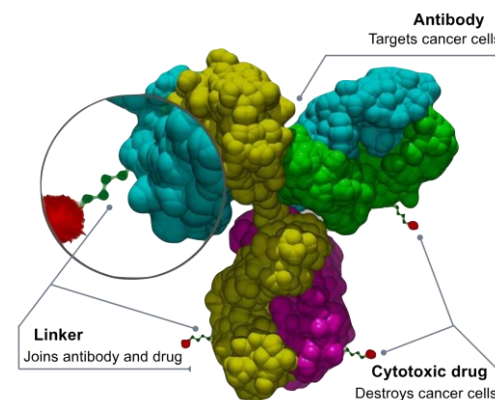
customizable selection process
*epitope-/conformation-specific
neutralizing antibodies*

improvable after selection
tunable stability/affinity

molecular engineering
*scFv, Fab, IgG, bispecifics, CAR-T,
site-specific conjugation (ADC)*



Annu. Rev. Med. 67, 165-83 (2016)



By Bioconjugator - Own work, CC BY-SA 4.0,
<https://commons.wikimedia.org/w/index.php?curid=58772304>



Brinkmann and Kontermann, 2017, mAbs

Rec Abs advantages for diagnostics and research

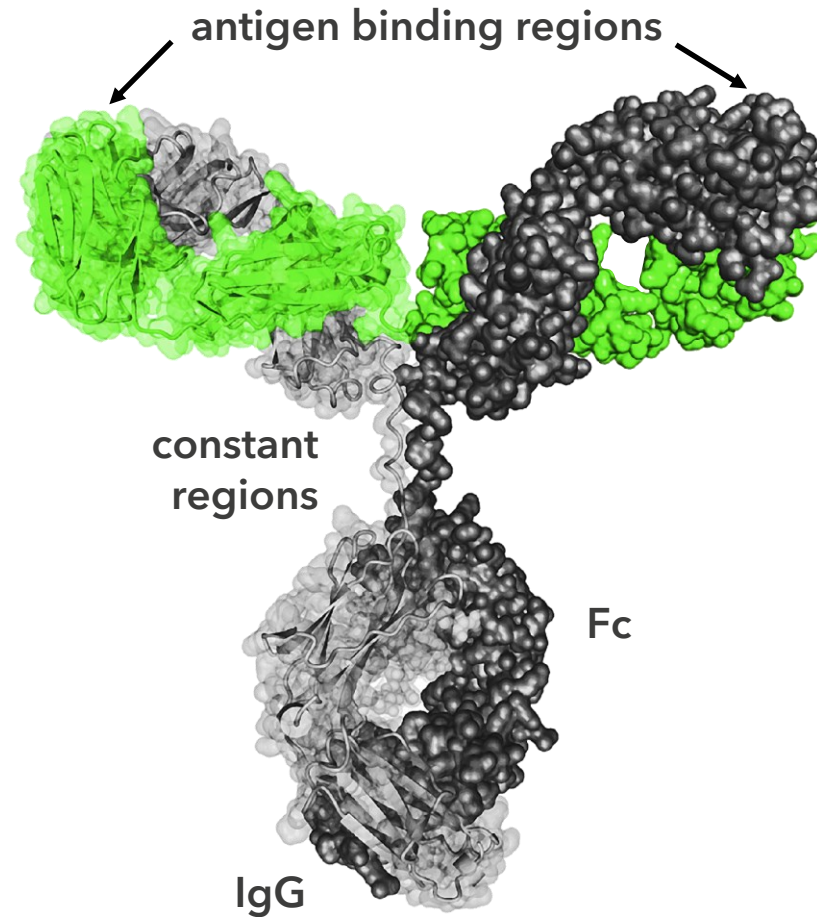
animal free generation
from human naïve libraries

sequence defined reagent

customizable selection process

improvable after selection

molecular engineering



Also beneficial for
non-therapeutic
applications?

Rec Abs advantages for diagnostics and research

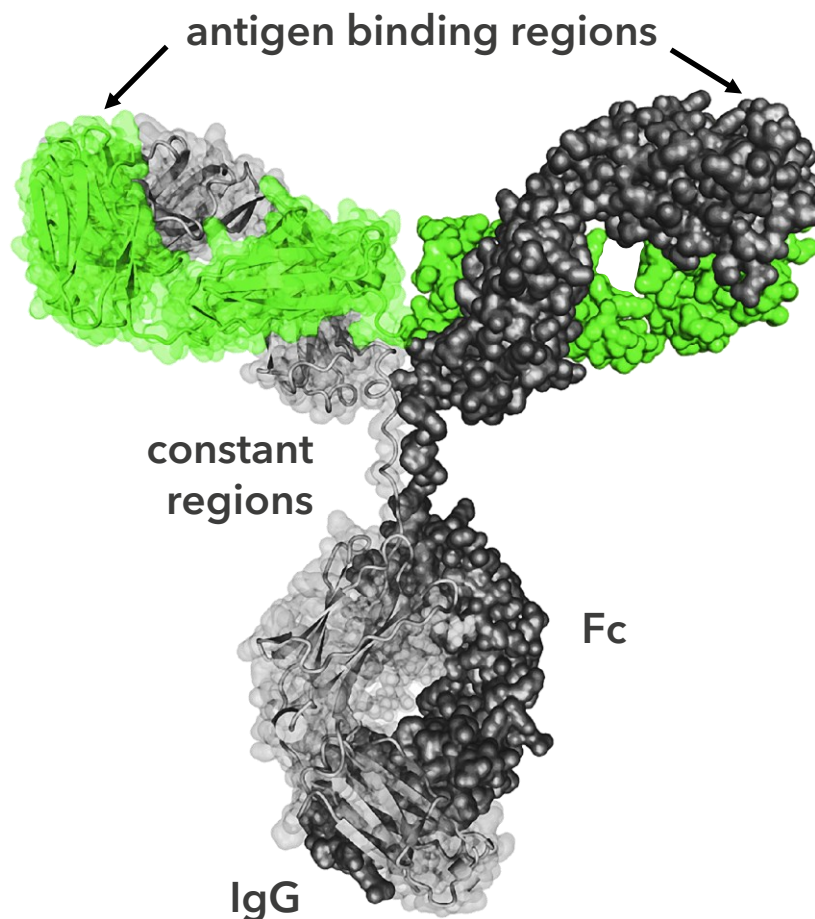
animal free generation
from human naïve libraries
reduce animal farming

sequence defined reagent
*unlimited reproducibility
and known composition*

customizable selection process
*negative selection of
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molecular engineering
free choice of format/Fc-part



Also beneficial for
non-therapeutic
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YES

Rec Abs advantages for diagnostics and research

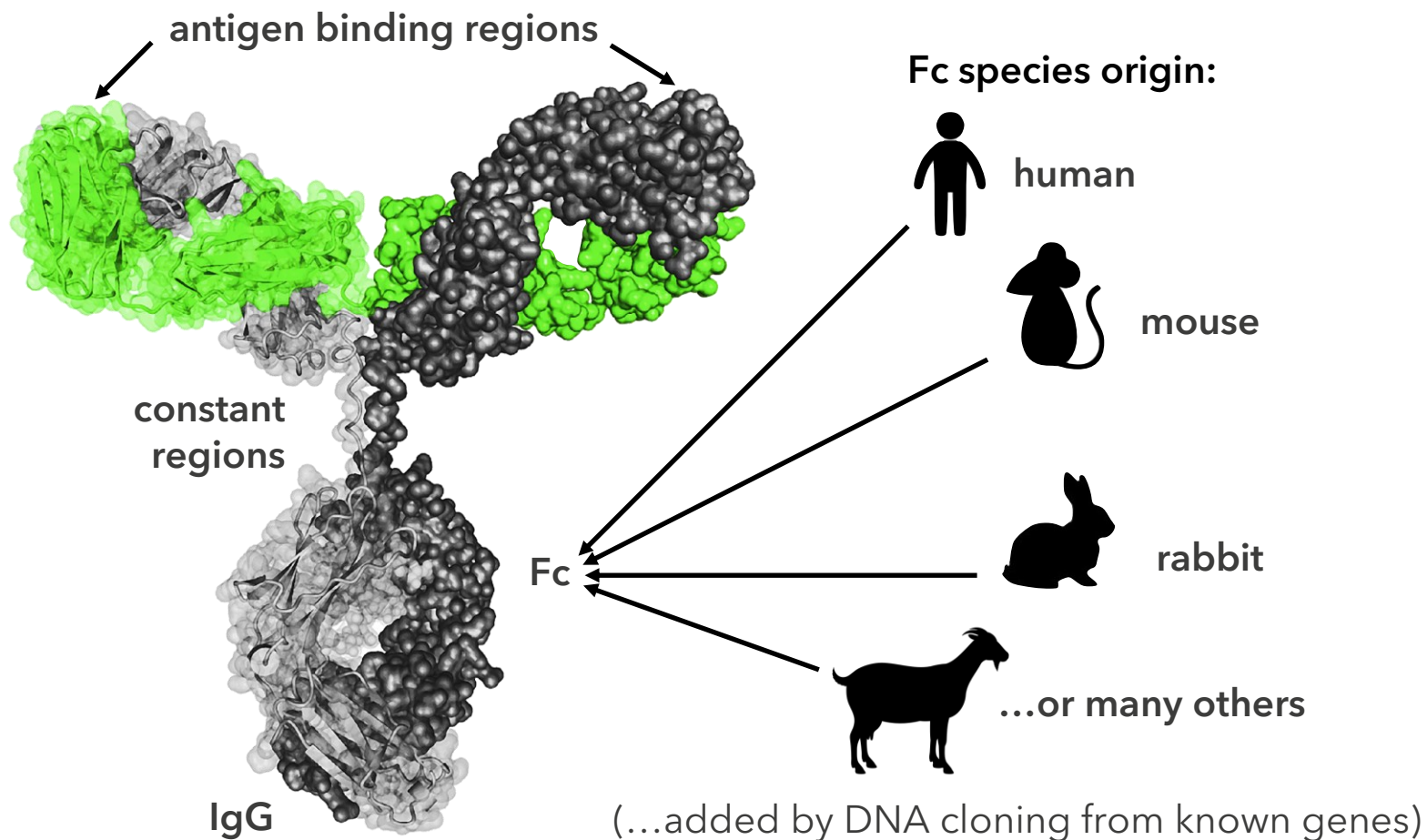
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Why non-therapeutic recAbs are not so common?

- conservatism: animal derived Ab generation established for >40 years
- unawareness of today's technology and existing high quality examples
- unawareness of the various additional opportunities of recAbs
- unawareness of existing commercial sources

Mission



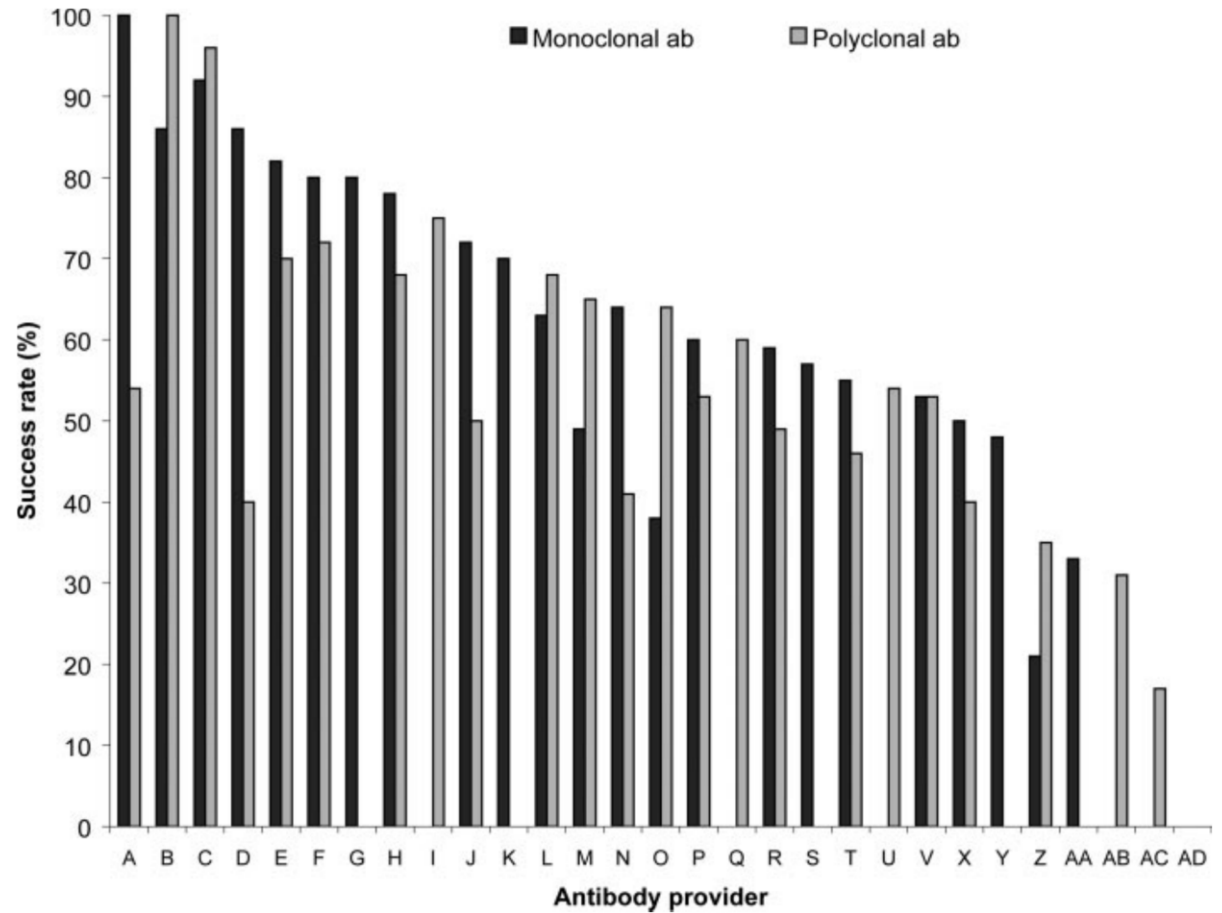
Abcalis aims at offering a concrete alternative to animal-based diagnostic reagents introducing thoroughly characterized, animal-free, sustainable and sequence-defined recombinant Mono- and Multi-clonal antibodies generated with the phage display technology.

Problem: animal derived antibodies

More than 5000 Abs from 51 different providers tested via Western Blot and IHC on tissue microassays.



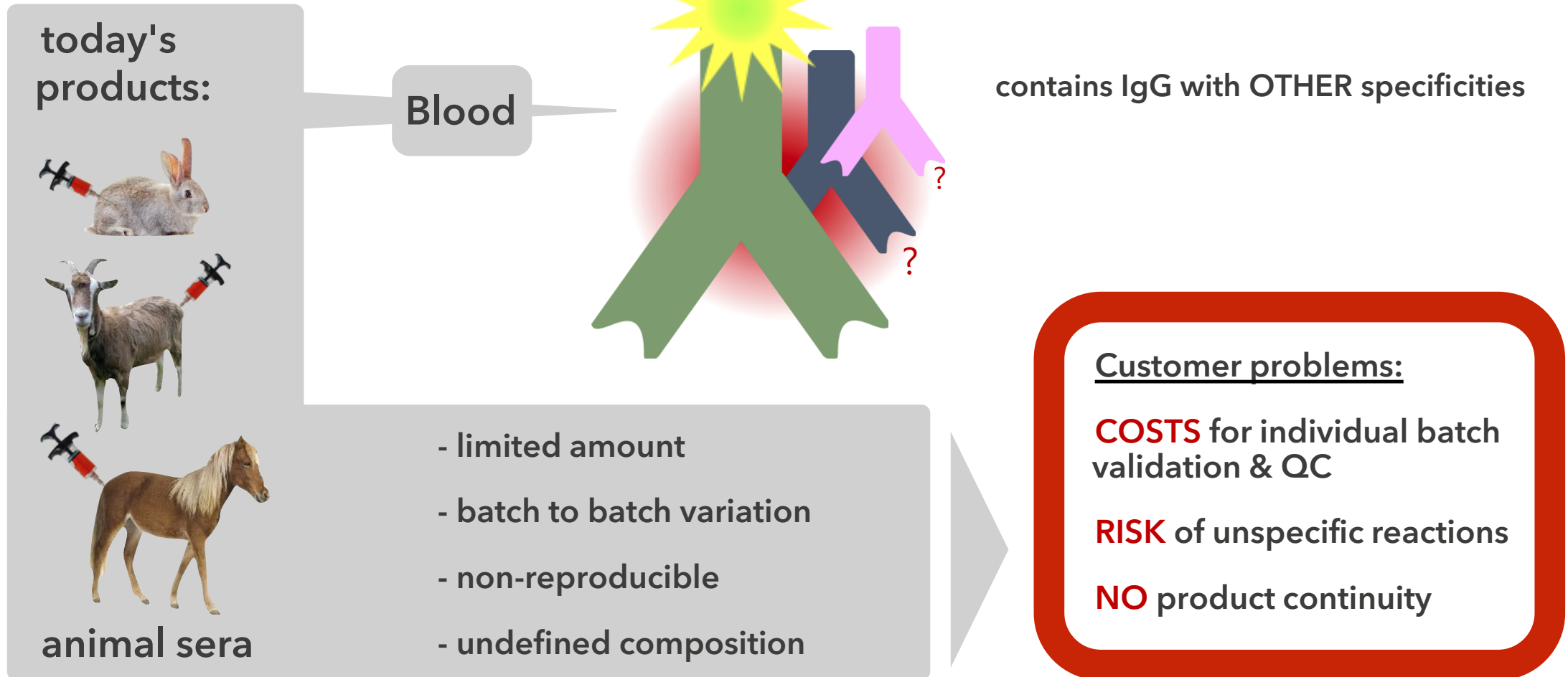
Less than 50% showed a staining pattern that is consistent with literature or bioinformatics data!



Berglund, L. et al., 2008, Cell Proteomics

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Animal polyclonals

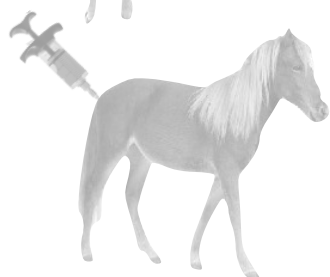


Gefördert durch:

Recombinant MULTICLONALS



today's
products:



animal sera



sequence
defined
recAb's

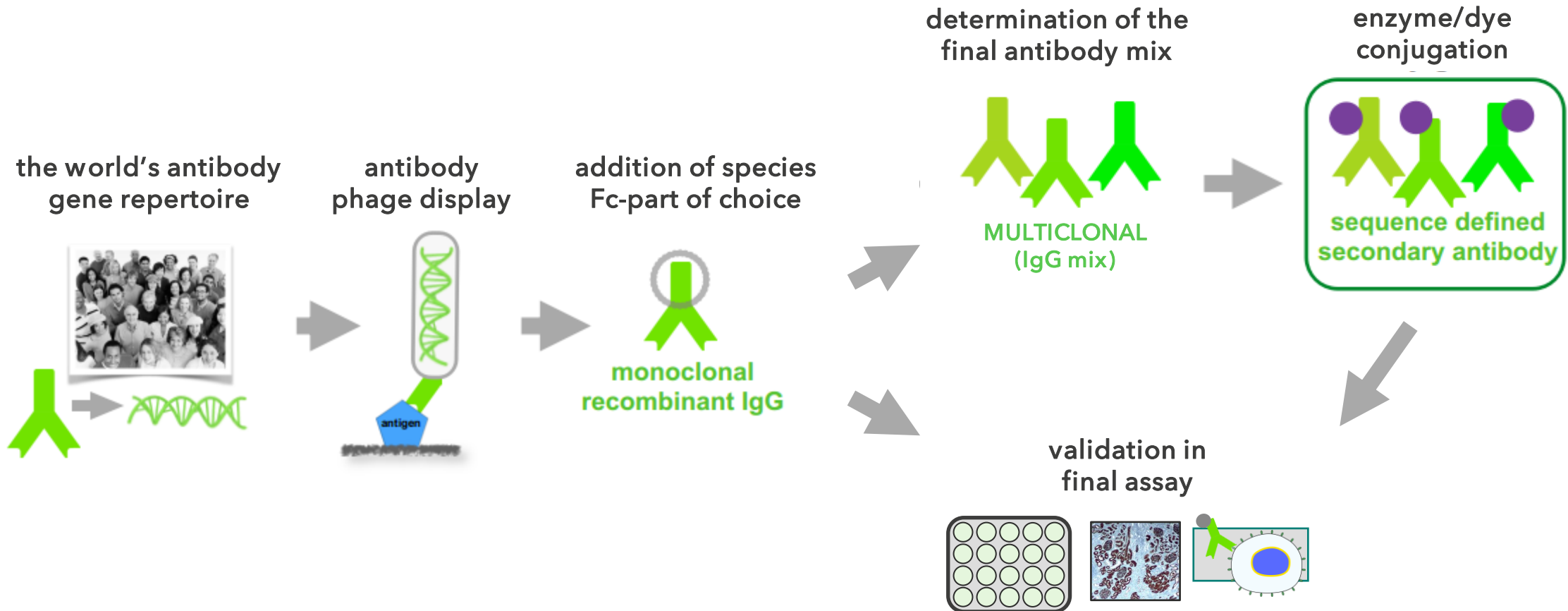
Abcalis recAb:



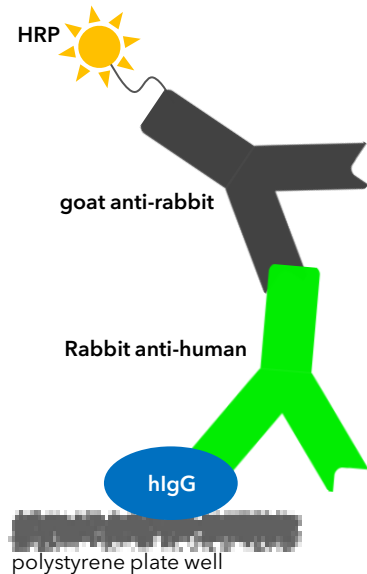
- ✓ animal-free
 - ✓ unlimited batch size
 - ✓ reproducible
- = Product continuity

- NO batch to batch variation
- FULLY DEFINED composition
- ANY TARGET possible
- SCALABLE production

Technology

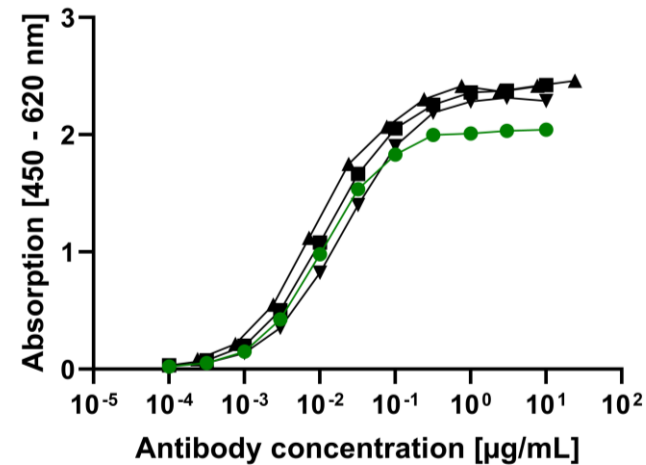


When is multi-clonality determinant?



Indirect ELISA set-up

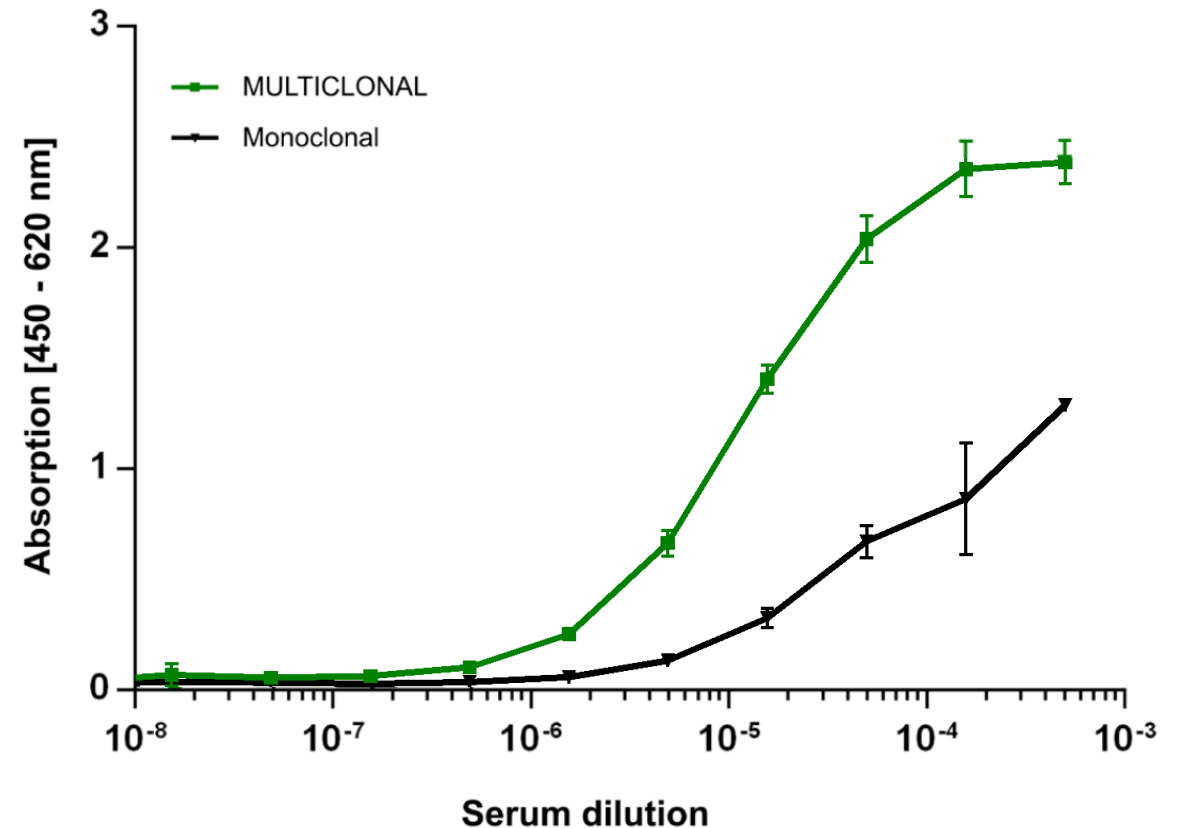
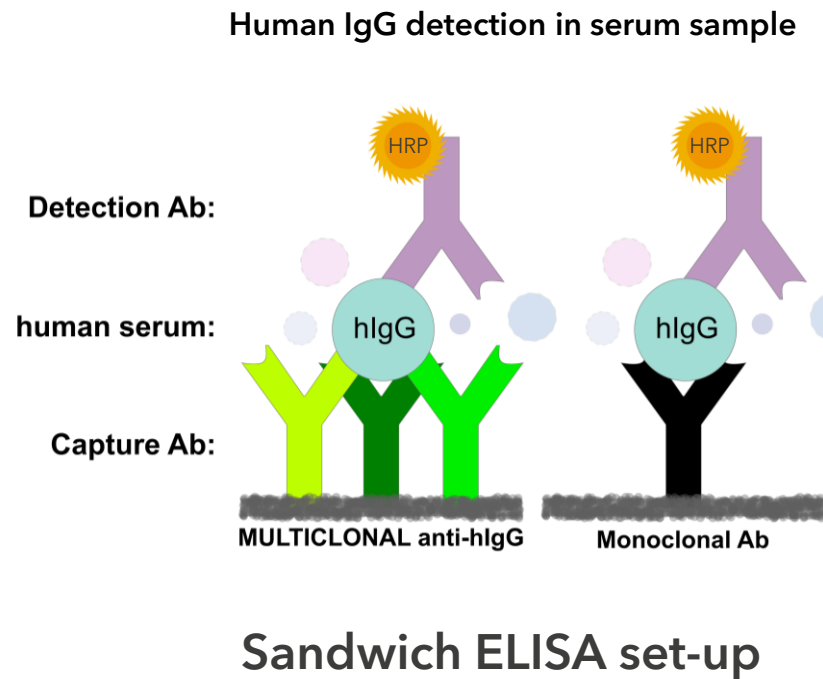
Rabbit anti-human Abs binding to coated hIgG



Anti-human antibodies:

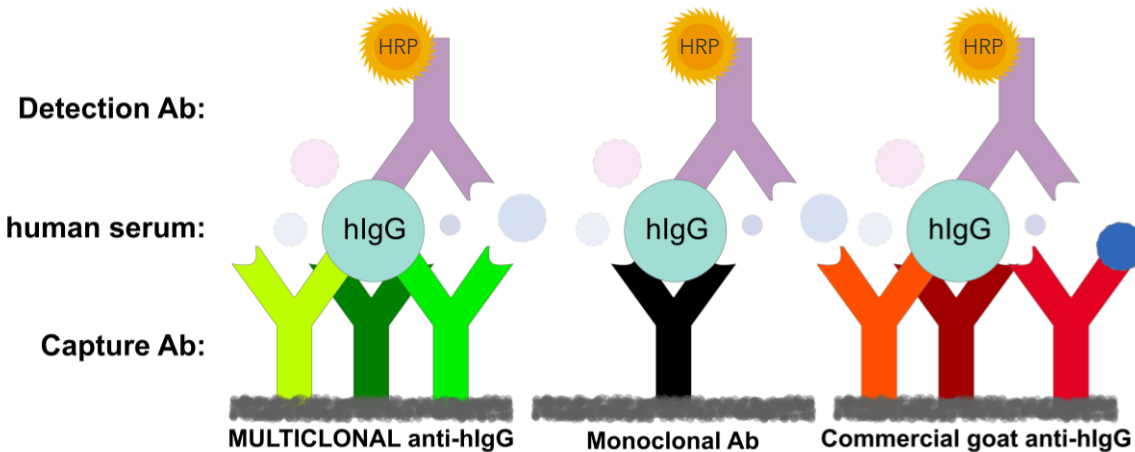
- Rab rec. Monoclonal IgG (SH1844-E3)
- Rabbit Polyclonal 1
- ▲ Rabbit Polyclonal 2
- ▼ Rabbit Polyclonal 3

When is multi-clonality determinant?

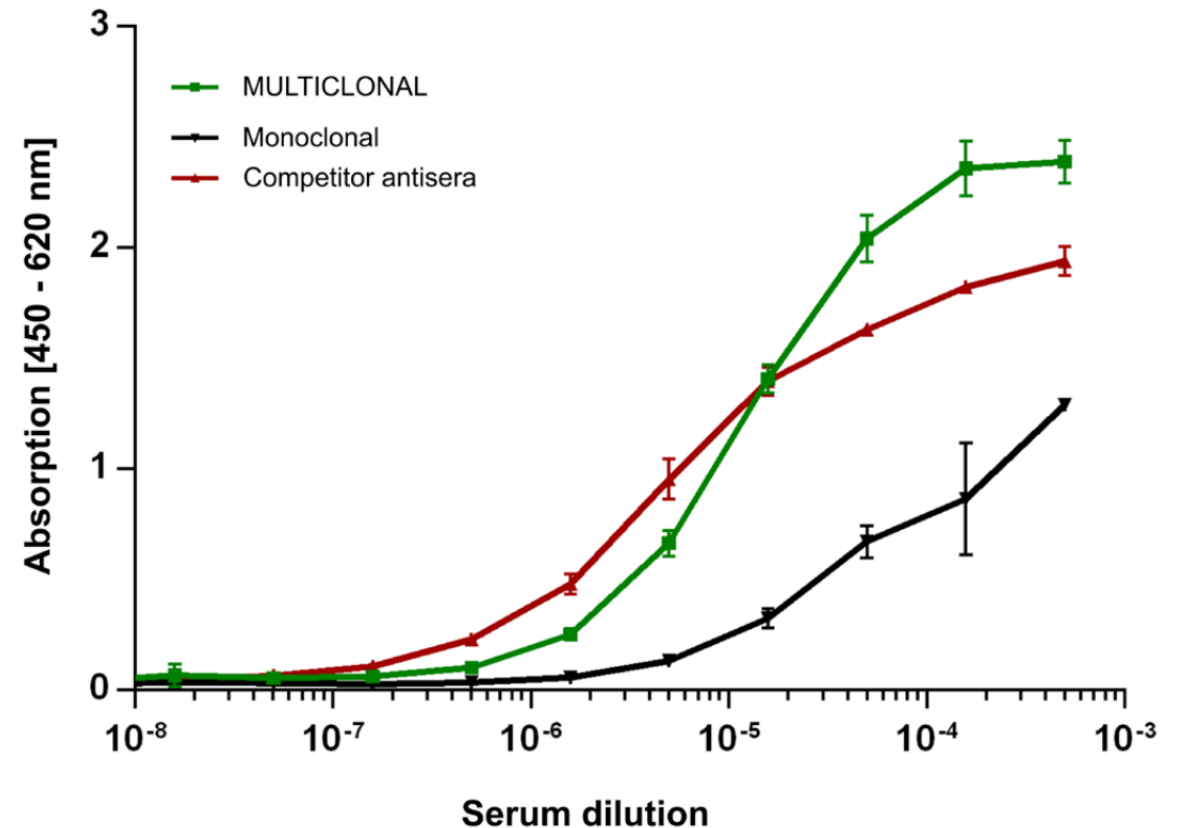


When is multi-clonality determinant?

Human IgG detection in serum sample

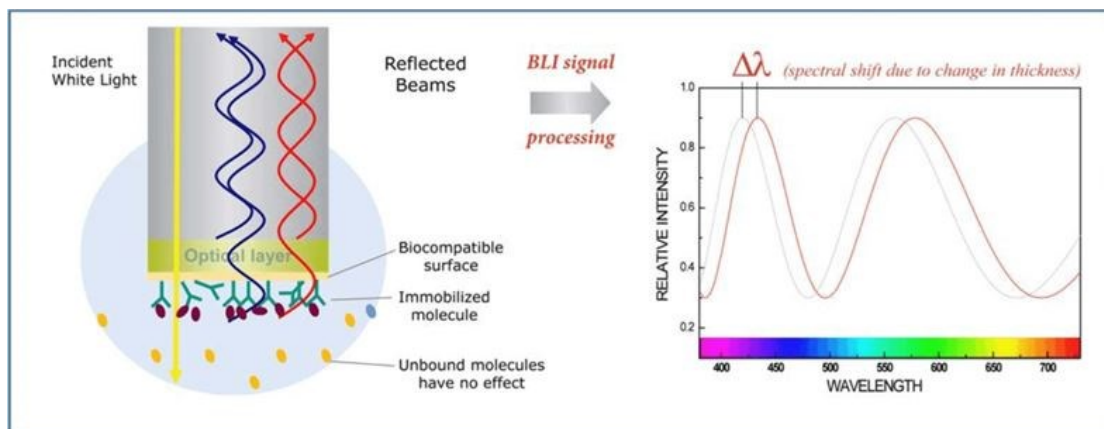


Sandwich ELISA set-up



MULTICLONAL assembly

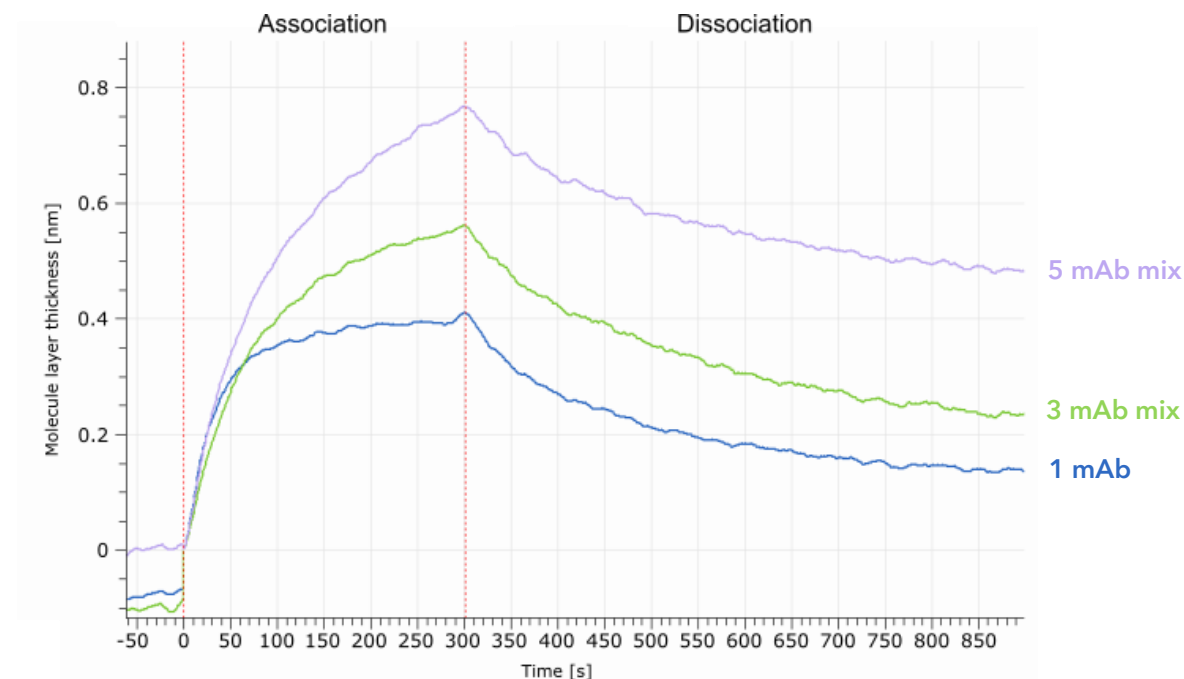
System: Forté-Bio Octet Qke (BLI)
Antigen Loading: 9 $\mu\text{g/ml}$ hlgG1 for 120 s
Ab concentration: 50 nM



<https://www.creative-biolabs.com/Octet-system.html>

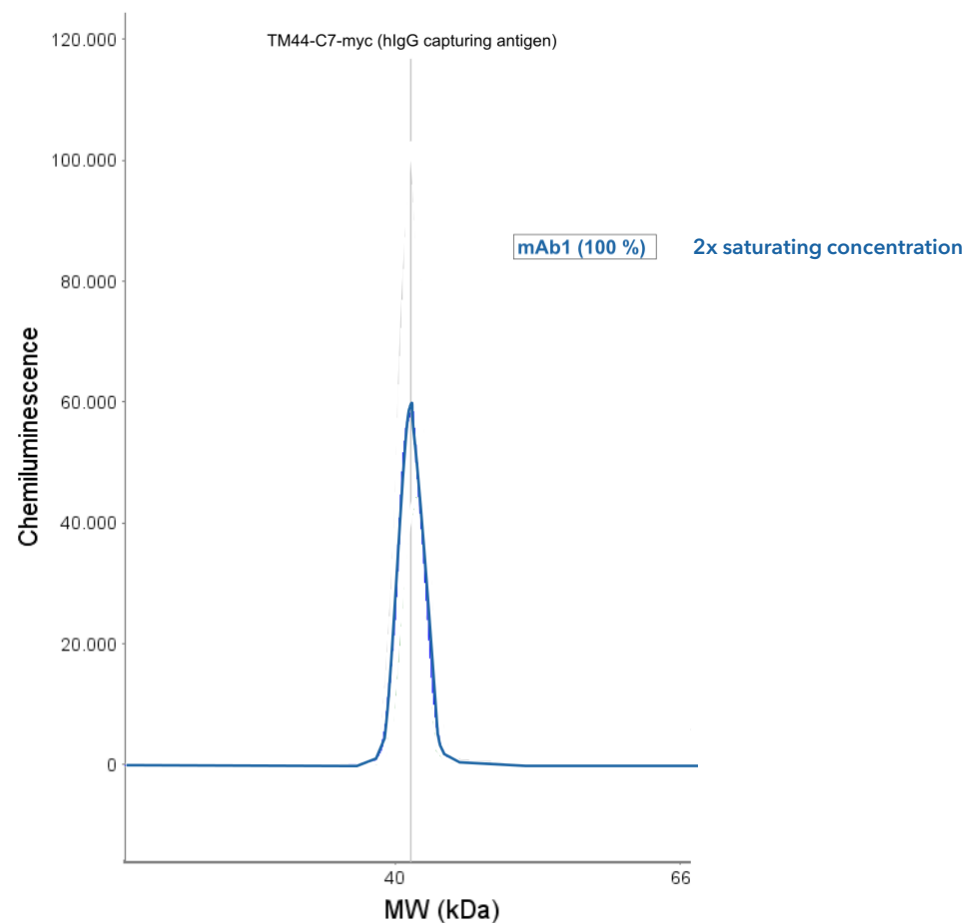
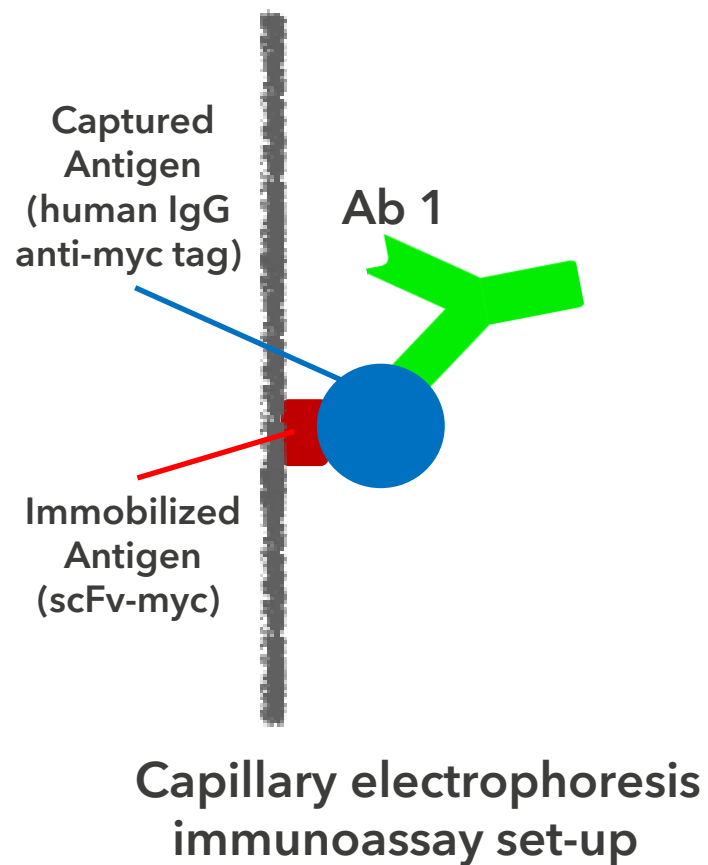
Biolayer interferometry molecular interactions and kinetics measurement

RecAbs binding to sensor immobilized hlgG

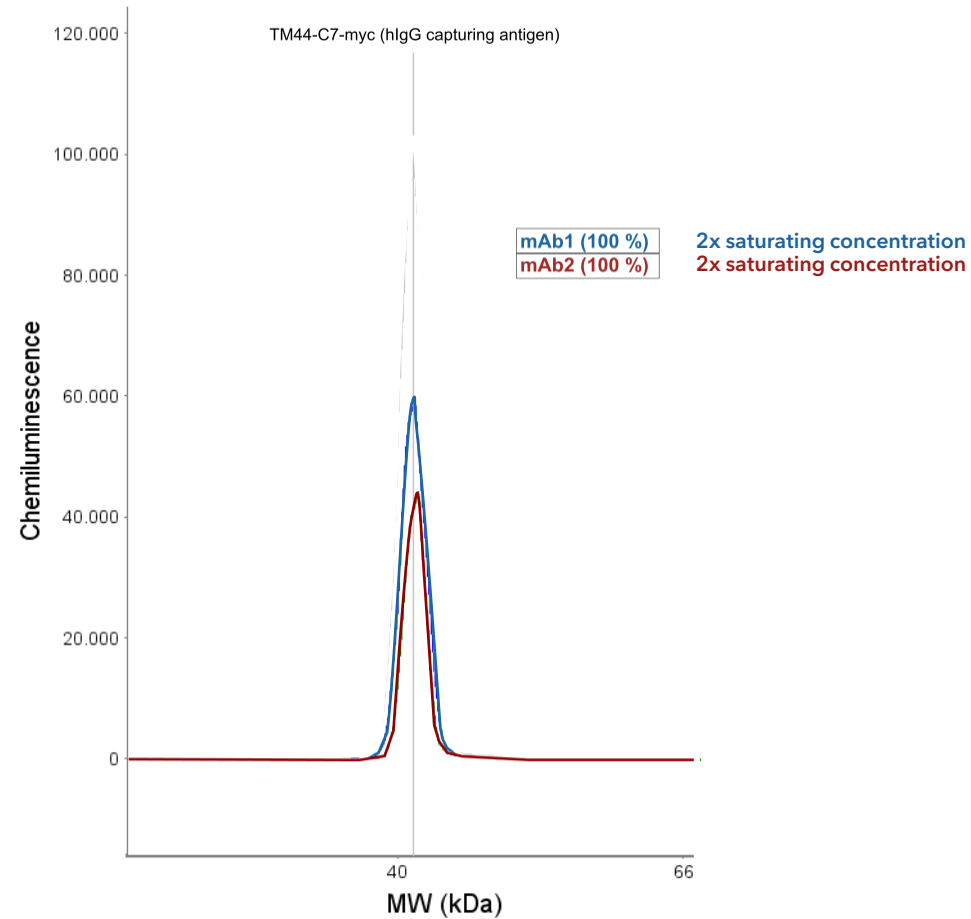
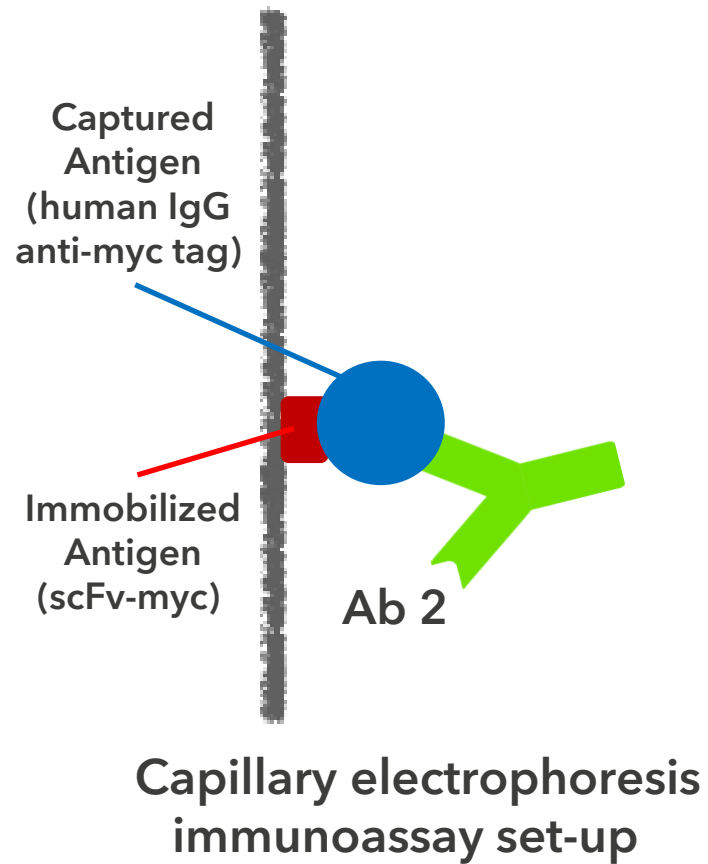


Gefördert durch:

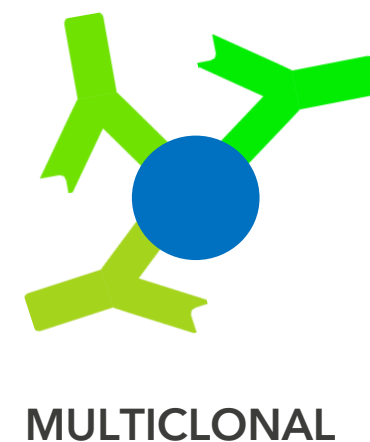
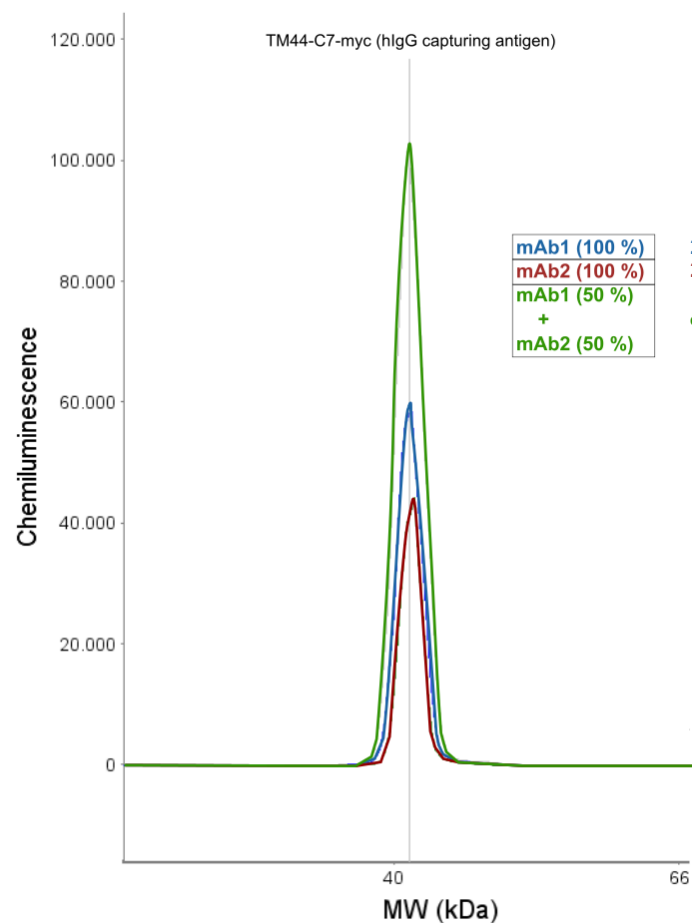
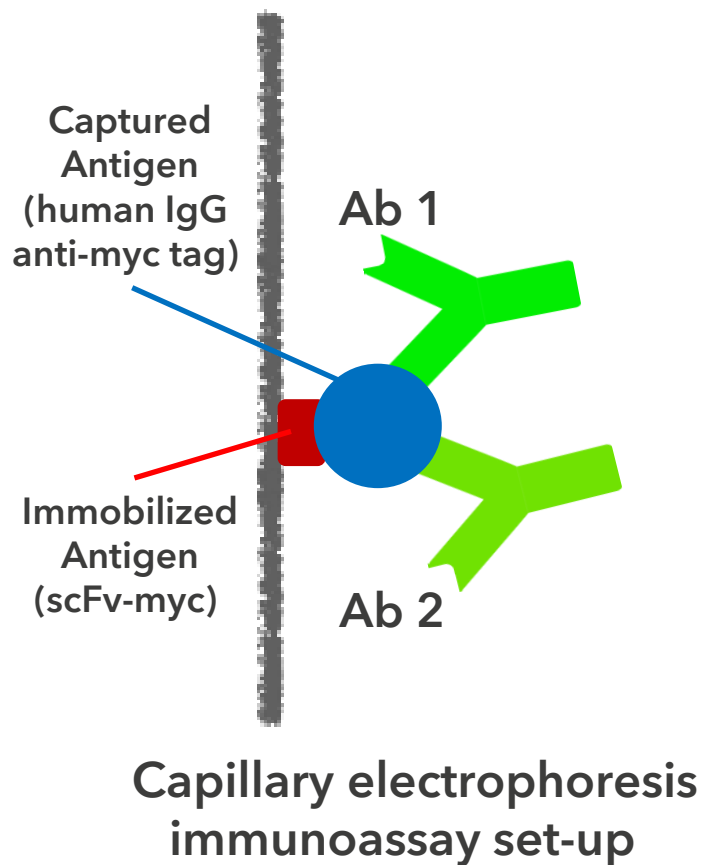
MULTICLONAL assembly



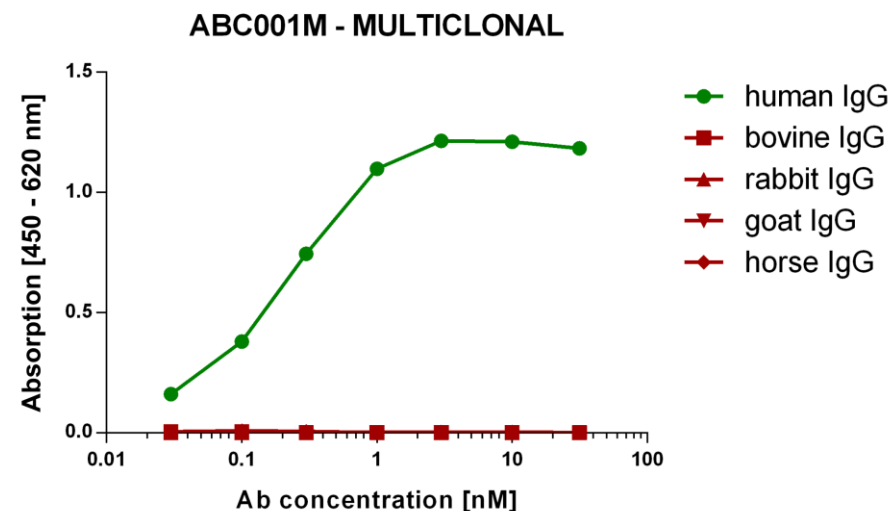
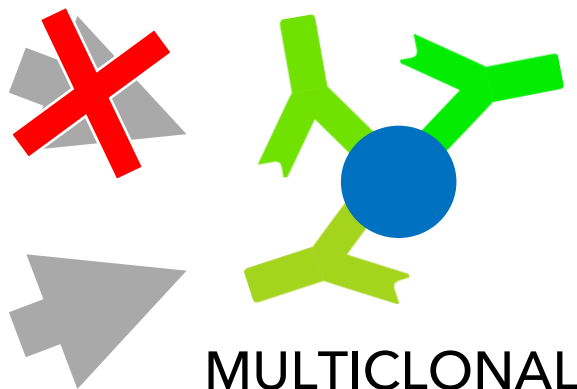
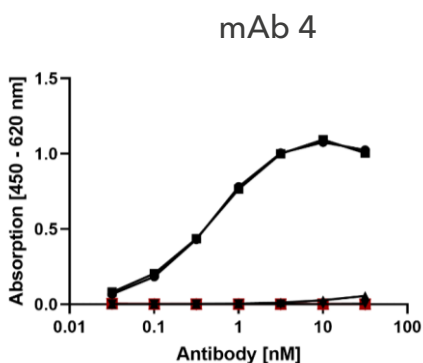
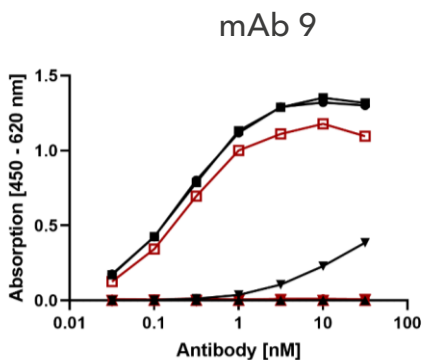
MULTICLONAL assembly



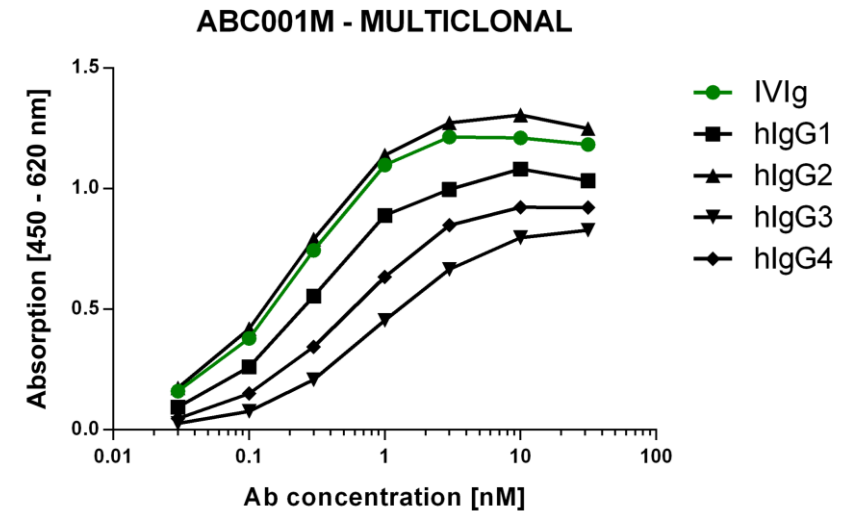
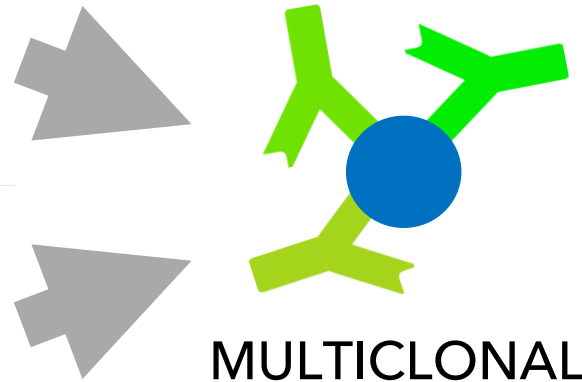
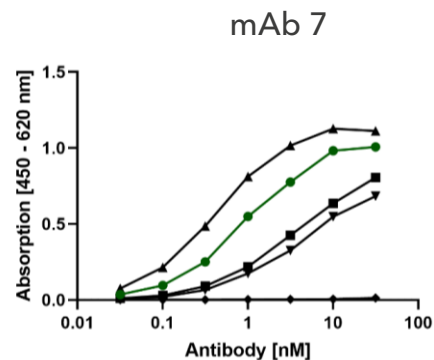
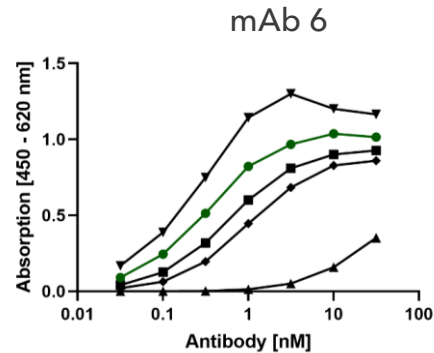
MULTICLONAL assembly



MULTICLONAL species specificity



MULTICLONAL subclasses specificity



Conclusion



<https://abcalis.com/multiclonaals/>

Recombinant MULTICLONAL antibodies are a concrete alternative to animal derived polyclonal antibodies!

Rec Abs advantages for diagnostics and research

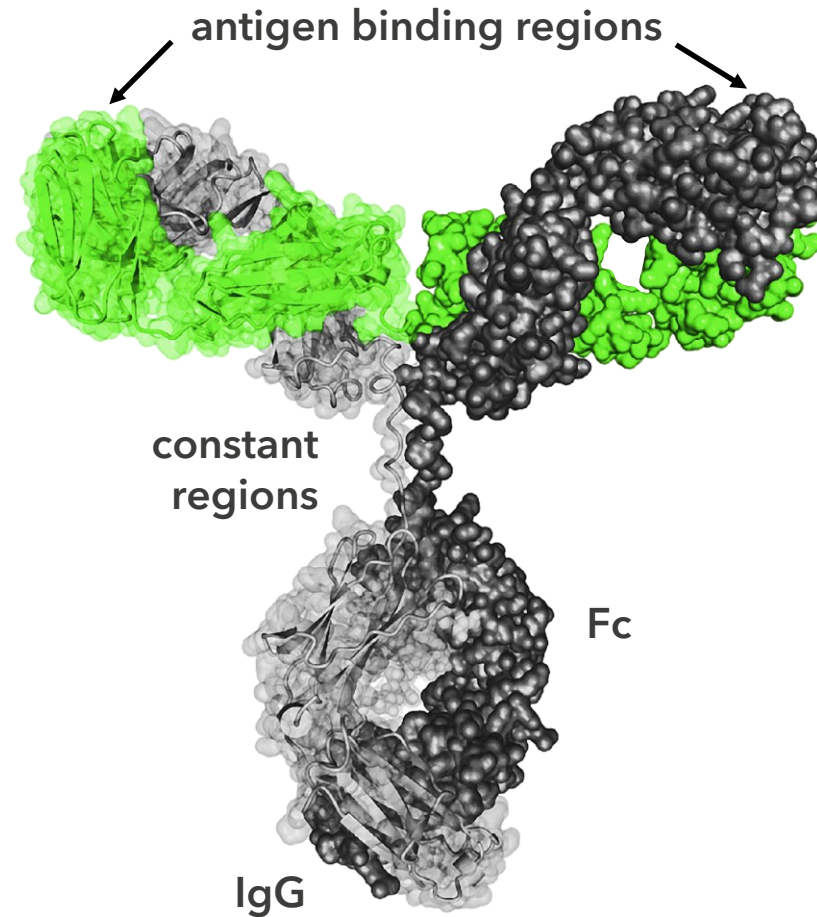
animal free generation
from human naïve libraries
reduce animal farming

sequence defined reagent
*unlimited reproducibility
and known composition*

customizable selection process
*negative selection of
unwanted reactivities*

improvable after selection
tunable stability/affinity

molecular engineering
free choice of Fc-part



IMMORTAL

Rec Abs advantages for diagnostics and research

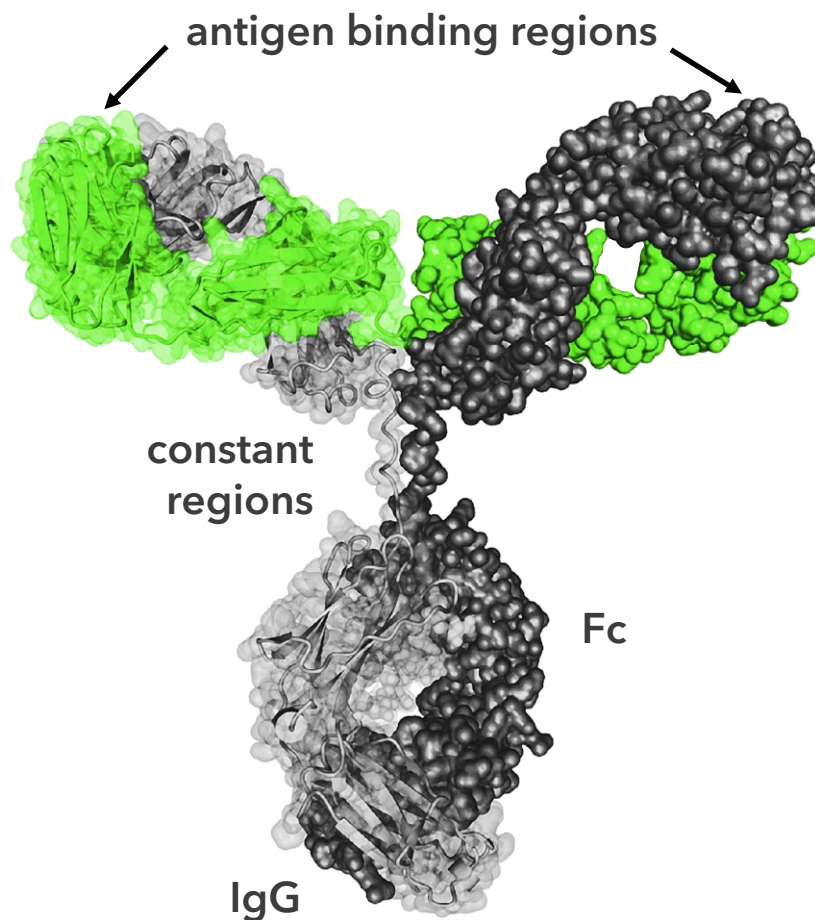
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IMMORTAL
CUSTOMIZABLE

Rec Abs advantages for diagnostics and research

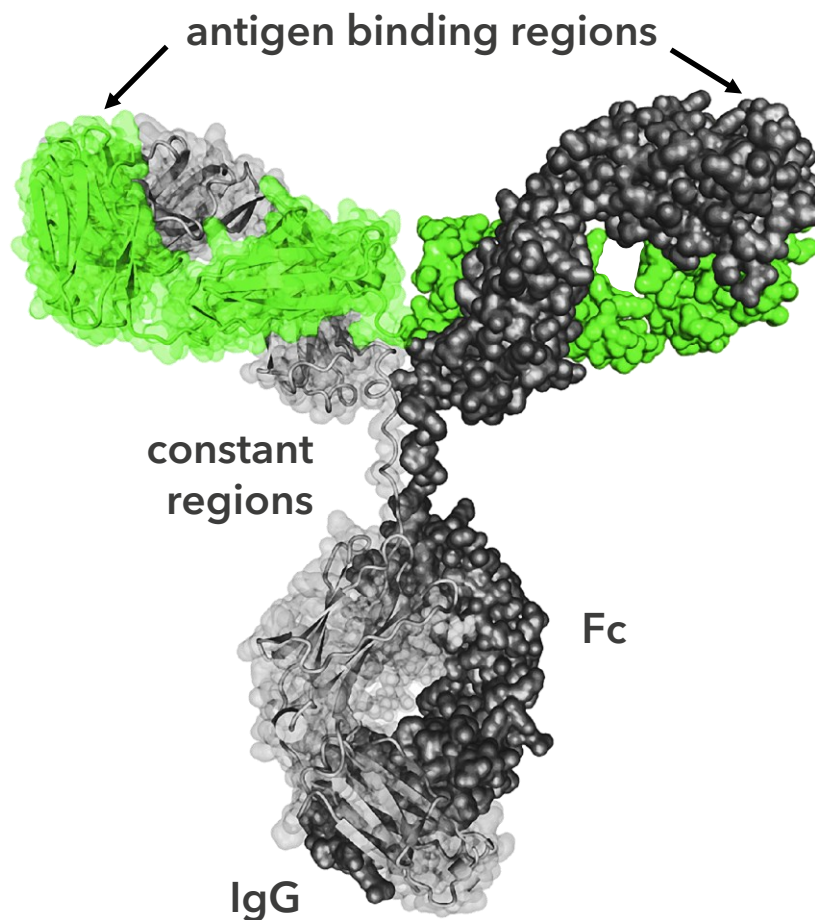
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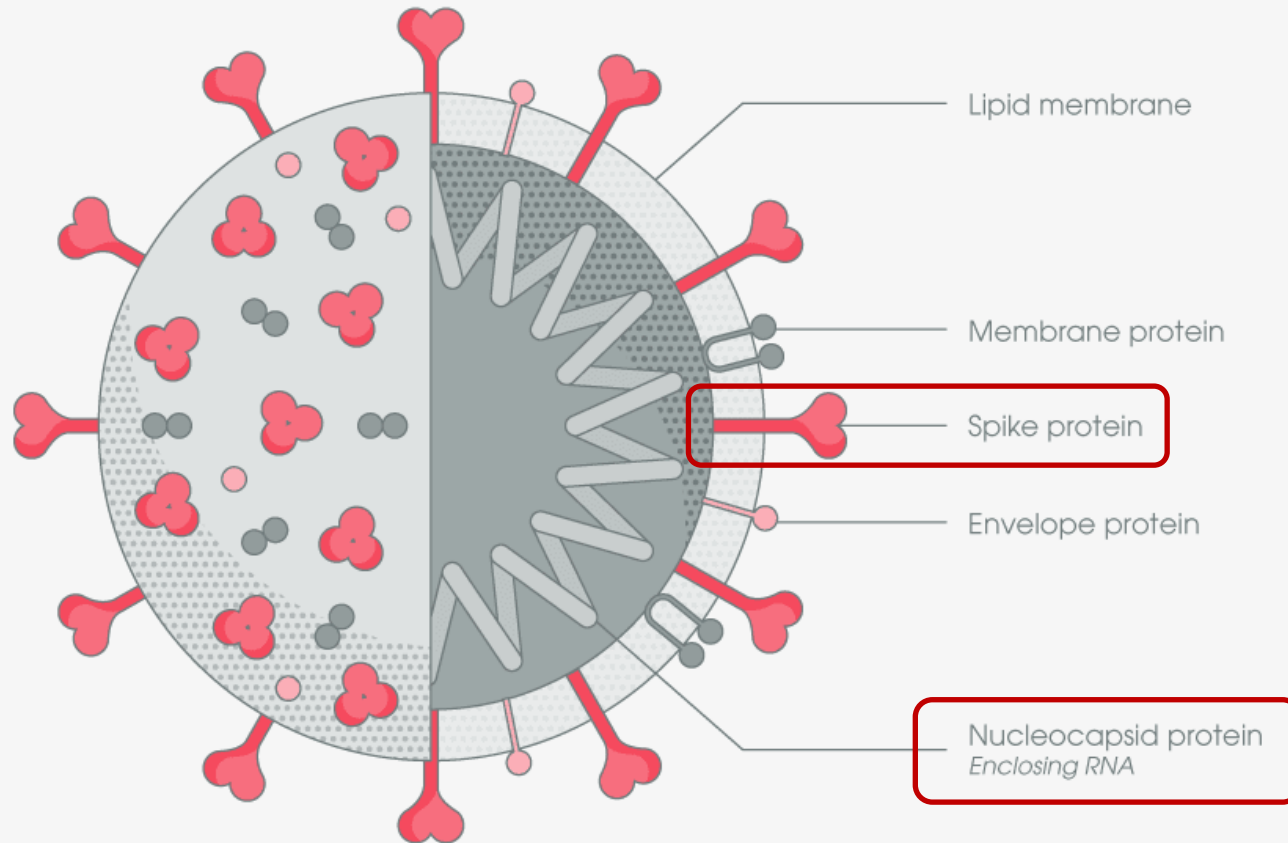


**IMMORTAL
CUSTOMIZABLE**



**Encourages
thorough
characterization**

COVID-19 Diagnostics

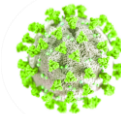


www.abcam.com/content/structural-and-functional-mechanism-of-sars-cov-2-cell-entry

>100 antibodies
tested against
SARS-CoV-2

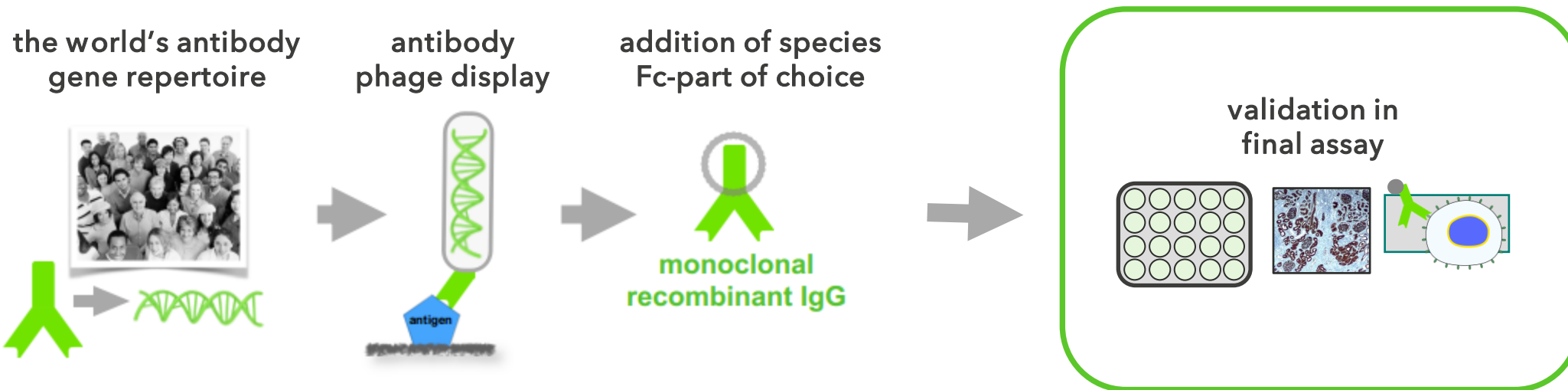
Applications:

- **Positive control in serological test instead of patient sera**
- **Virus detection in rapid antigen tests**



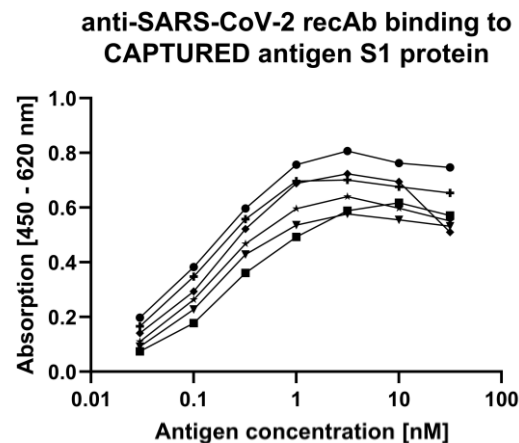
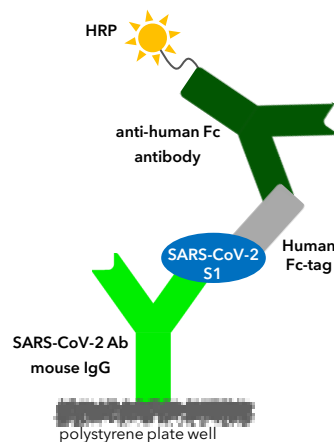
First tests ongoing!

anti-S1 SARS-CoV-2 clone ABC68 validation



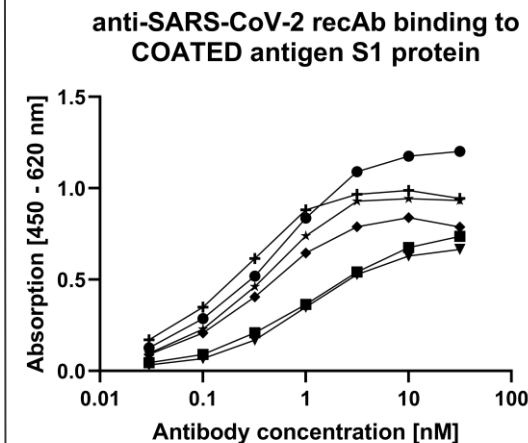
Binding in ELISA

Sandwich ELISA

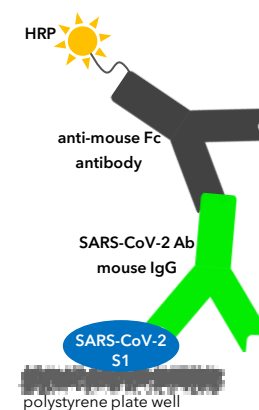


+ ABC54 + ABC75
 + ABC63 + ABC76
 • ABC68 + ABC82

Indirect ELISA



+ ABC54 + ABC75
 + ABC63 + ABC76
 • ABC68 + ABC82



Binding to S1 mutants

Specificity profile (different hcov)

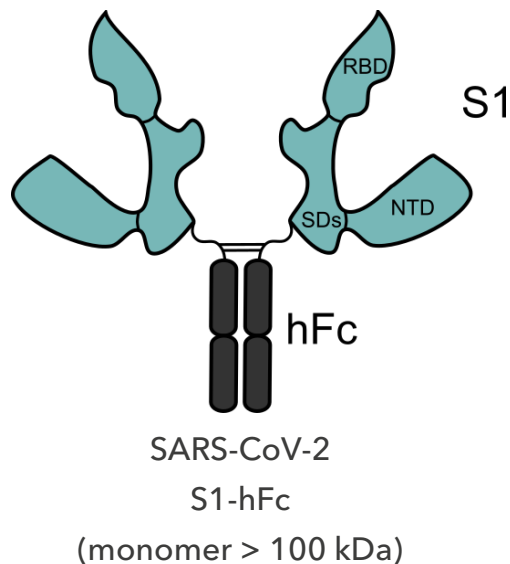
Storage stability and reproducibility

Binding to PFA fixed antigen (IF)

Binding to denatured antigen (WB)

Binding to soluble or plate-coated antigen (ELISA)

Denatured antigen binding



Binding to
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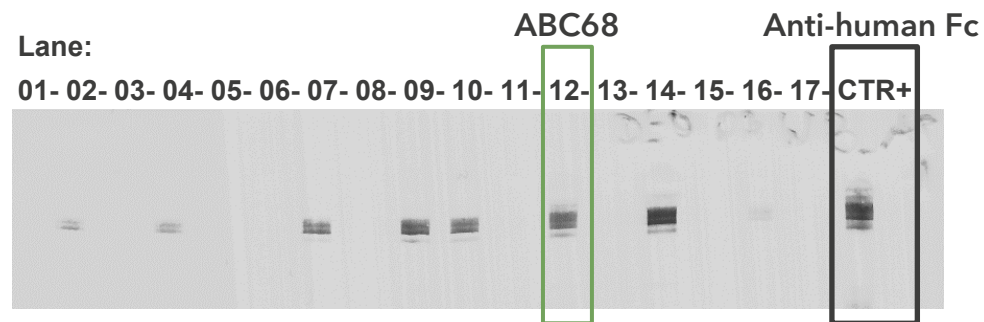
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Binding to PFA fixed antigen (IF)

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Western Blot



Antigen preparation:

Non-reducing conditions
SDS-PAGE protein separation
Ag. loading: 0.5 µg/lane

Detection:

Rec. mIgG anti-SARS-CoV-2 S1 -> 1 µg/mL
anti-mouse-AP conjugated

Antigen binding after fixation

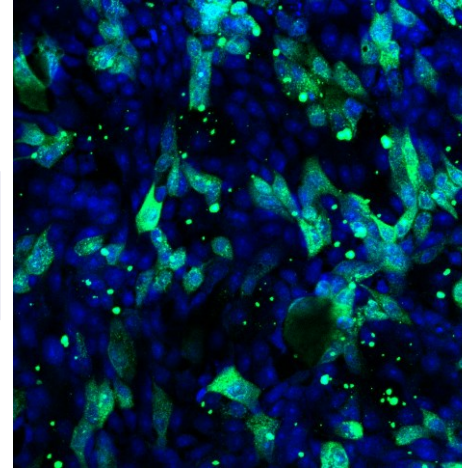
Immunofluorescence staining

Note:
cell fixation in 6% PFA 1h RT

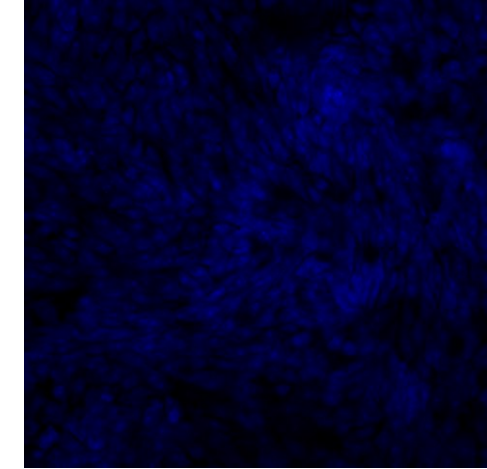
anti-SARS-CoV-2 S1
ABC68 (mIgG)
20 µg/mL

DAPI

Calu-3 cells infected with
SARS-CoV-2 (strain Zagreb)

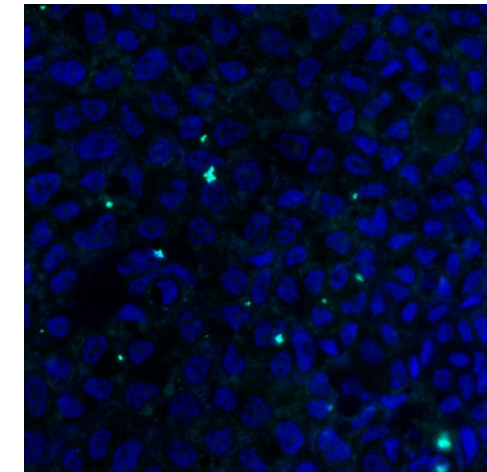
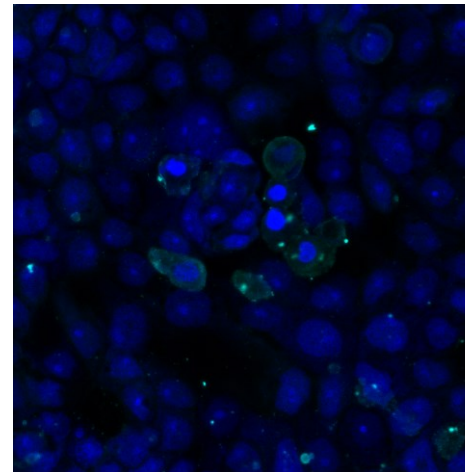


Calu-3 cells uninfected



anti-dsRNA
J2 Ab (mIgG)
20 µg/mL

DAPI



Data: Ulfert Rand, Luka Cicin-Sain, HZI

Gefördert durch:

Antigen binding after fixation

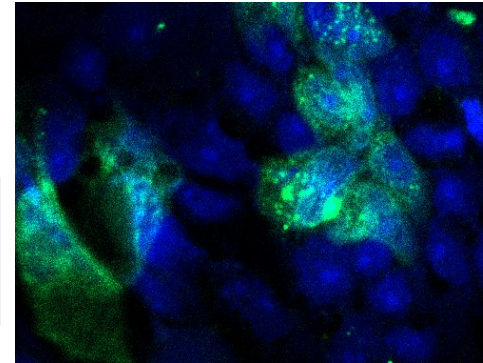
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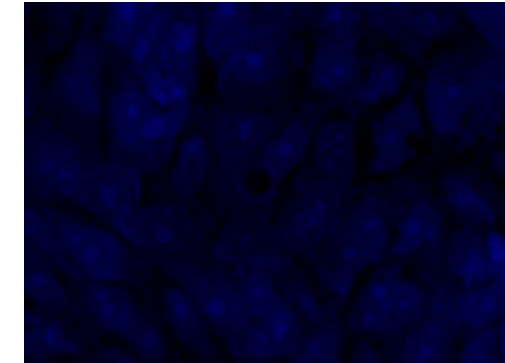
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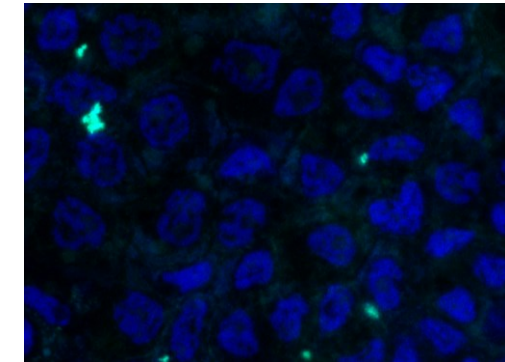
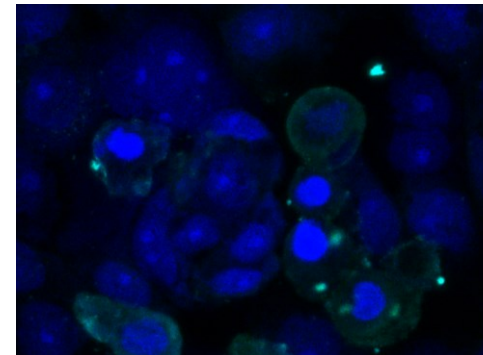


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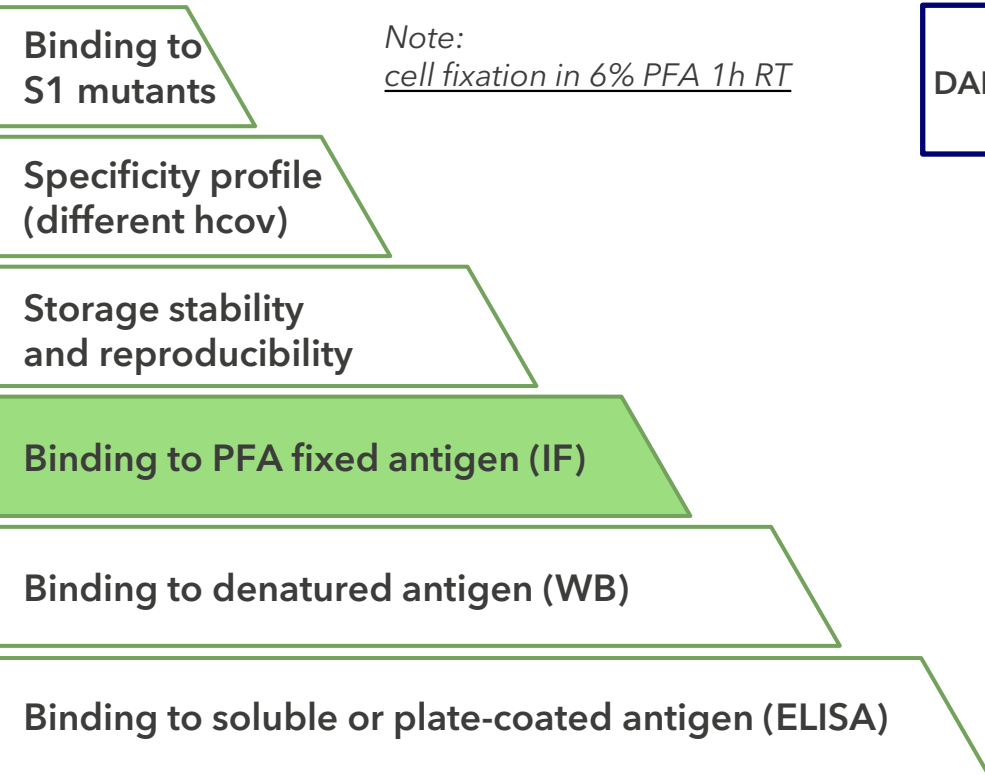


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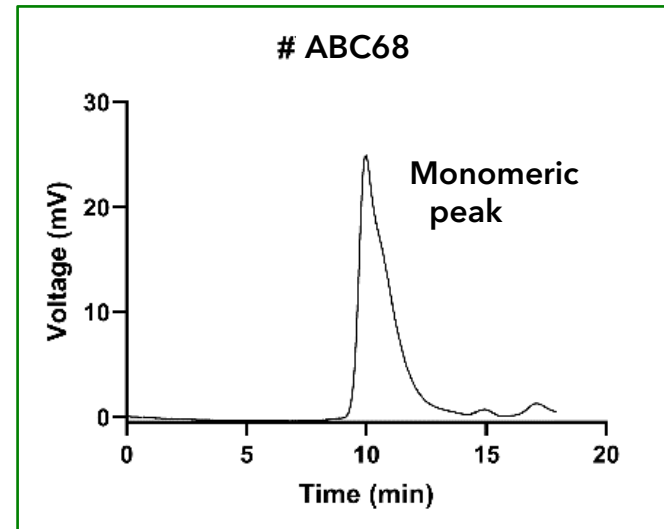
Data: Ulfert Rand, Luka Cicin-Sain, HZI



Antibody stability

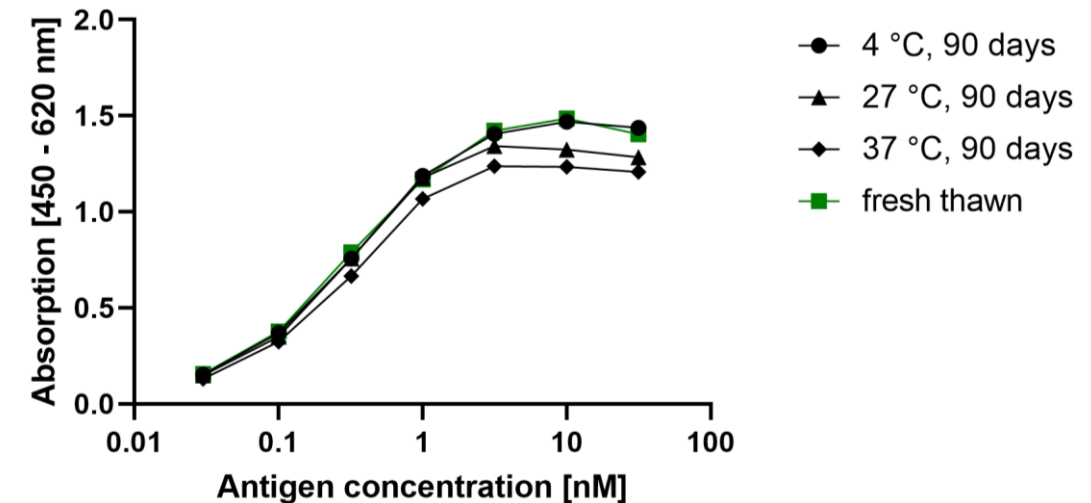
- Binding to S1 mutants
- Specificity profile (different hcov)
- Storage stability and reproducibility
- Binding to PFA fixed antigen (IF)
- Binding to denatured antigen (WB)
- Binding to soluble or plate-coated antigen (ELISA)

HPLC-SEC (size exclusion chromatography)



Separation column:
AdvanceBio SEC 300 A 2 7 μ m 7.8 x 150 mm, PL 1180 3301

mIgG ABC68 binding to S1-hFc after storage



Storage in phosphate buffer saline (PBS) pH 7,4
without stabilizers or protectants.

Batch-to-batch consistency



Transient production in mammalian and insect cells!

Binding to S1 mutants

Specificity profile (different hcov)

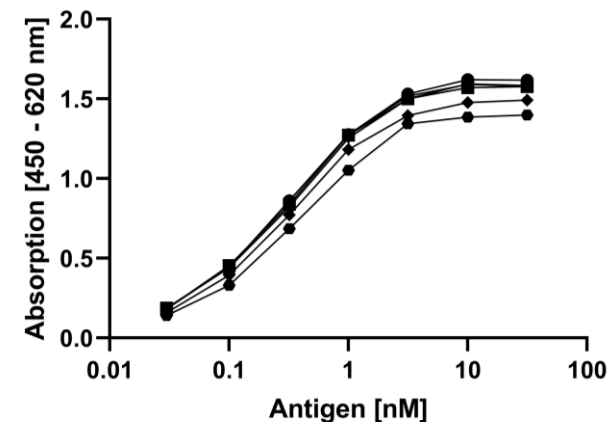
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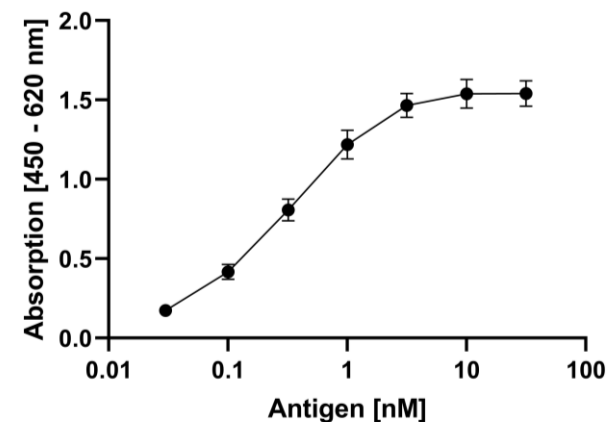
mlgG ABC68 binding to S1-hFc



Production ID:

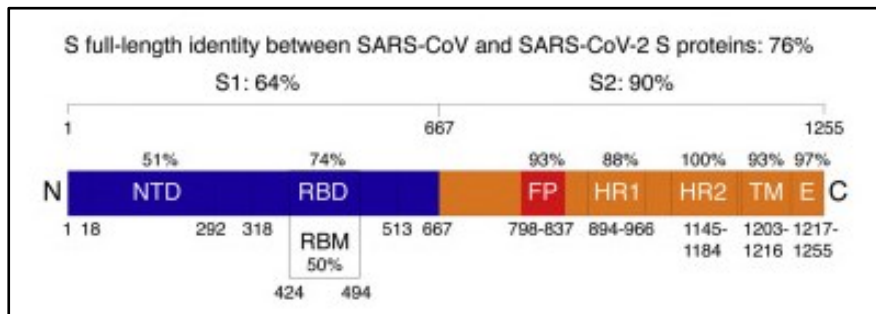
- YABC4-12
- YABC5-7
- ▲ YABC6-3
- ▼ YABC7-2
- ◆ YABC8-2
- YABC8-7

● Average signal



Gefördert durch:

Human coronaviruses cross-reactivity profile



Binding to S1 mutants

Specificity profile (different hcov)

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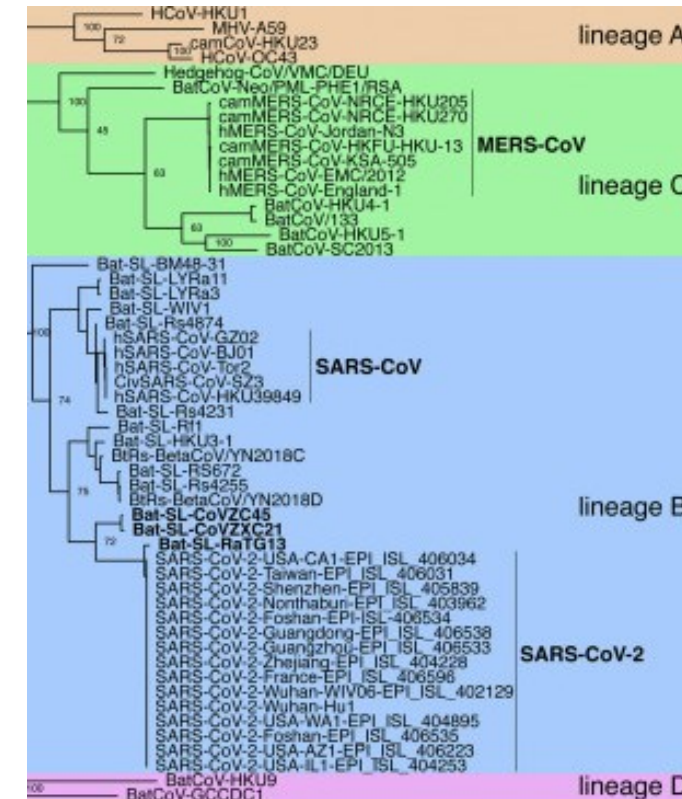
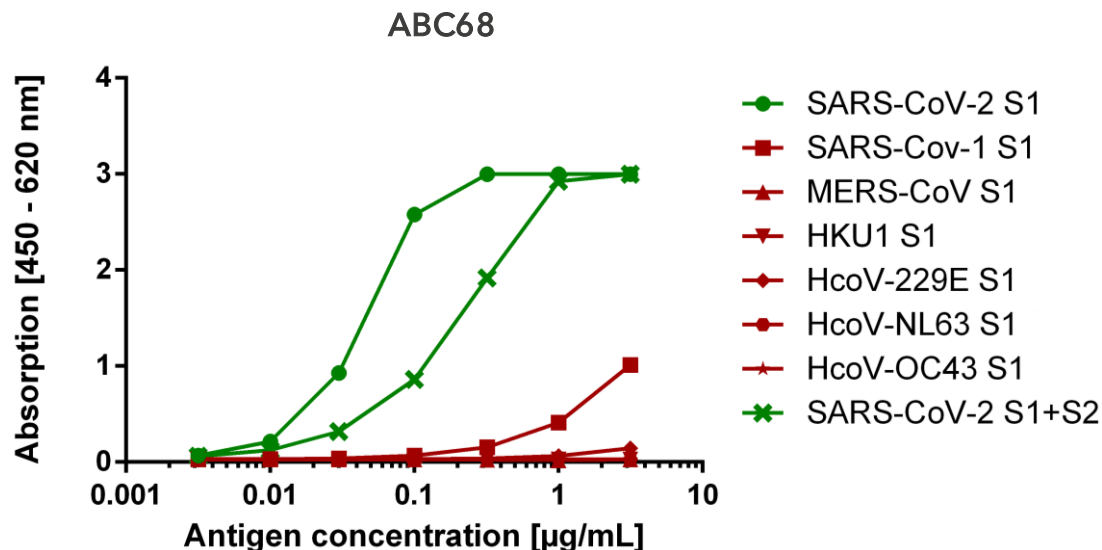


Jaimes et al., 2020, J Mol Biol.

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Jaimes et al., 2020, J Mol Biol.

SARS-CoV-2 mutants detection



(15 Oct. 2020)

SARS-CoV-2 identified mutations

 10416 Complete Genomes	 111 Sampling Locations	 82062 Total Mutations	 6678 Mutating Positions
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Rakha, A., Rasheed, H., Batool, Z., Akram, J., Adnan, A., & Du, J. (2020). COVID-19 Variants Database: A repository for Human SARS-CoV-2 Polymorphism Data. bioRxiv. <http://covid-19.dnageography.com/>

Binding to
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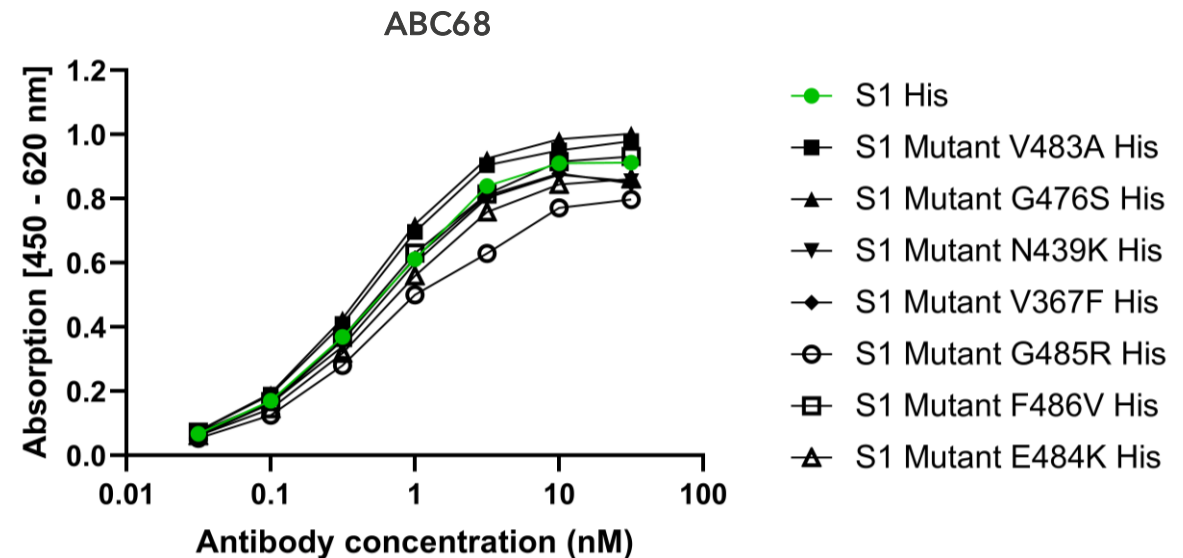
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Rec Abs advantages for diagnostics and research

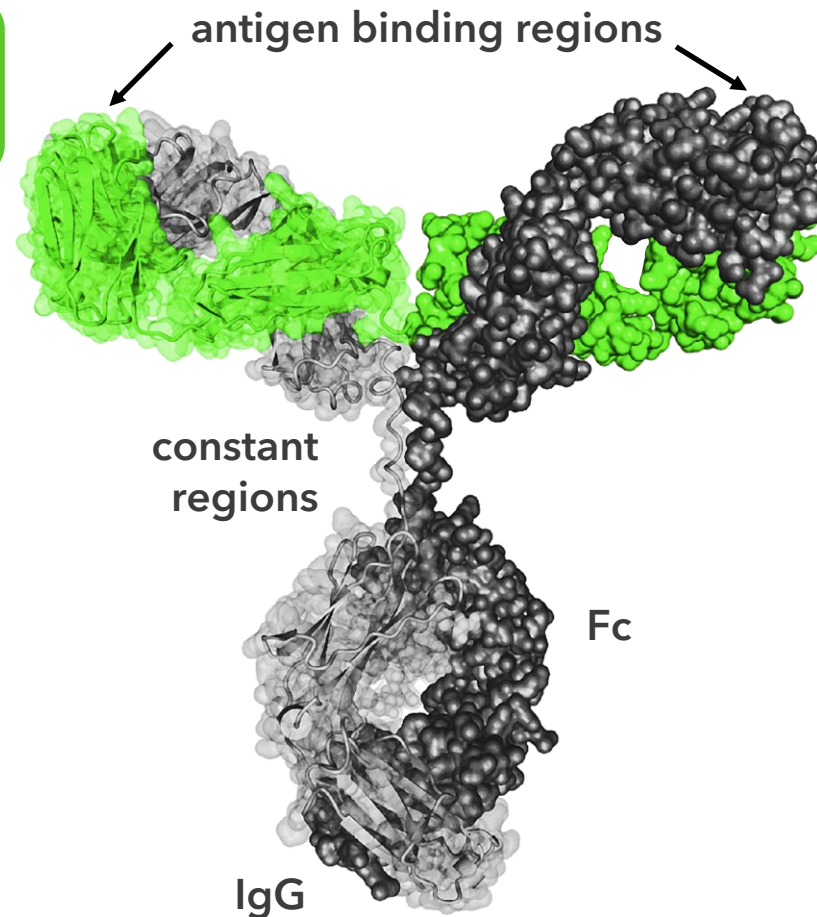
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IMMORTAL
CUSTOMIZABLE
SUSTAINABLE
SPECIFIC



Thanks to all the
cooperators and
funders!

Luka Cicin-Sain, HZI



Ulfert Rand



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