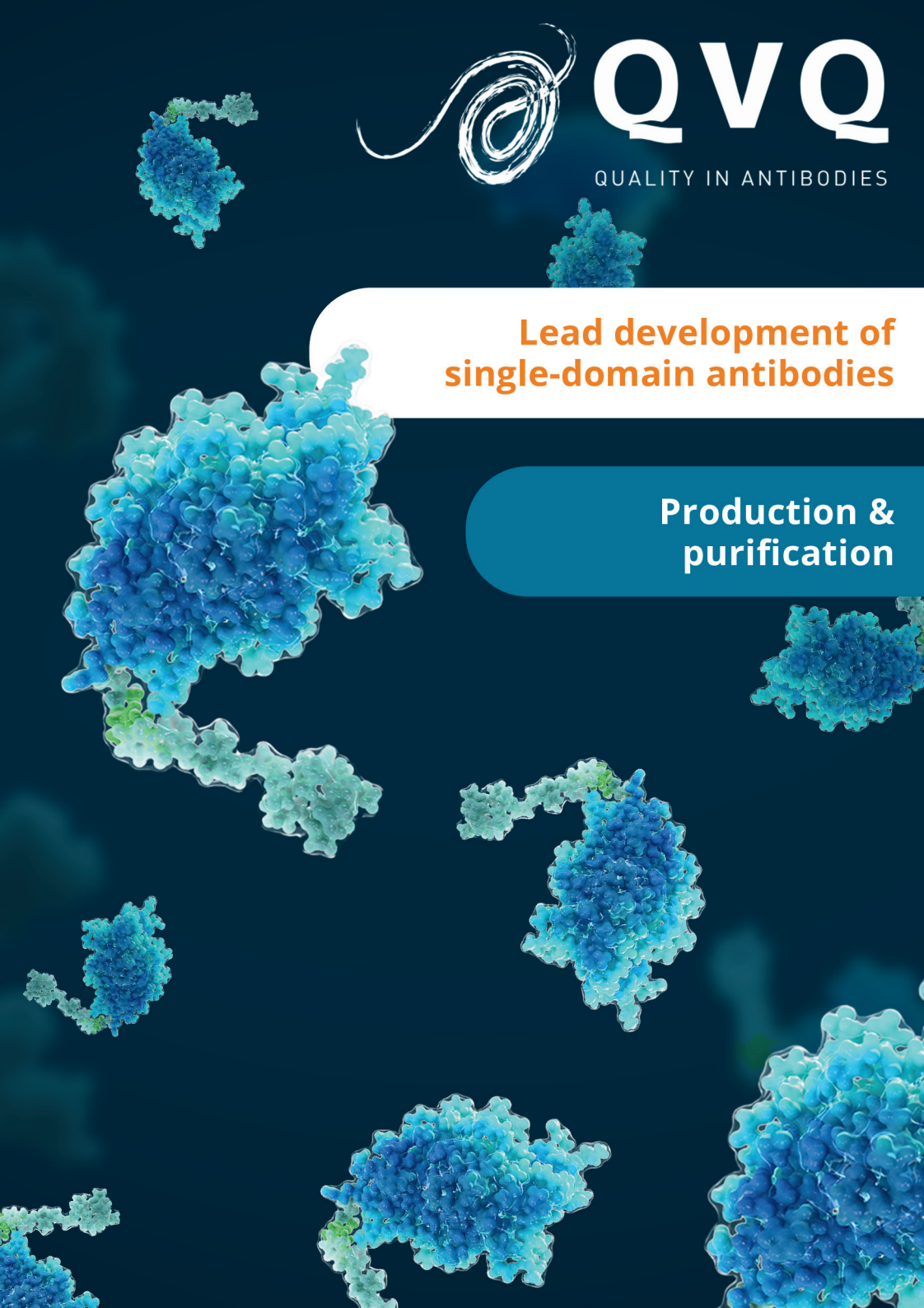
QVQ

QUALITY IN ANTIBODIES

Lead development of single-domain antibodies

Production & purification



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QVQ
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QVQ is your experienced partner for generating single-domain antibody panels

- Customized project plan
- Various sdAb libraries
- Goal-oriented panning
- Competitive & Cross-reactive binders
- NGS & Analyses
- Production & Purification
- Affinities & Kinetics
- Epitope binning
- sdAb pharmacology
- Engineering & Optimization

Full IP ownership

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Content of this booklet

Single-domain antibodies derived from camelid heavy-chain-only antibodies

Single-domain antibodies

Single-domain antibodies (sdAbs) are the variable domains of heavy-chain-only antibodies, naturally found in animals of the camelid family (such as camels, llamas, and alpacas) (Figure 1). These sdAbs are approximately ten times smaller than conventional antibodies and, also because of their uniquely shaped paratope, offer several advantages.[1,2] These include superior tissue penetration, the ability to bind deep within protein clefts, rapid clearance from the body (can be made undone upon desire), high stability, cost-effective production, and ease of reformatting.[3–6] As a result, sdAbs are ideal targeting molecules for a wide range of applications, from simple detection assays to advanced drug delivery systems.[6–8] Various sdAbs formats have already been clinically validated.[6,9]

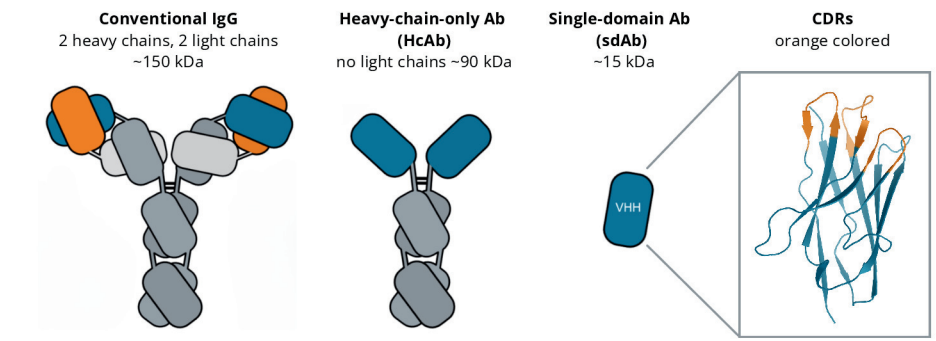


Figure 1. Structure of a sdAb. A conventional antibody composed of two heavy chains and two light chains which need each other for stability (left). A heavy-chain-only antibody lacks light chain but remains stable (middle). A sdAb is the isolated binding domain of a heavy-chain-only Ab (right).

As one of the pioneers in the field, QVQ offers expertise in the technology of each step in sdAb discovery. This is used to obtain the desired epitope, stability or specificity for your lead panel. QVQ offers full intellectual property rights and excellent value for money.

QVQ has extensive expertise in the generation and characterization of single-domain antibodies. As part of our services, QVQ provides custom lead development projects which are tailor made for each customer, based on our broad range of services. A full project from until the delivery of clones can be performed within five months (Figure 2).

Single-domain antibodies derived from camelid heavy-chain-only antibodies

Services include:

- Immunization of llamas and/or alpacas
- Preparation of VHH-phagemid libraries
- Ready-to-use VHH-phagemid libraries (immune, non-immune, synthetic, combinatorial)
- Customized selection procedures by phage-display biopanning
  - On natural or recombinant proteins (with e.g. His tag, Fc-tag), peptides (conjugated to e.g. KLH, ovalbumine, albumin) and more complex samples like cells and tissue
- Screening monoclonal sdAbs for the desired characteristics using ELISA and/ or flow cytometry
- Production and purification of sdAb, plain or equipped with different tags, in bacteria, yeast, or mammalian cells
- Quality control and validation of purified sdAbs

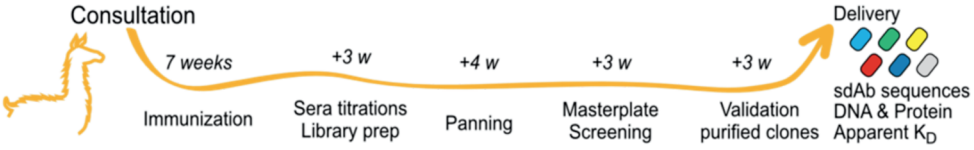


Figure 2. Timeline of a standard lead development project.

Optional Follow-up services include:

- Next-generation sequencing of outputs
- Characterization (ELISA, Flow cytometry, SPR)
- Larger scale production and purification
- Custom functionalization and labeling
- Optimization and humanization
- sdAb pharmacology

References:
1 Lameris et al. (2014) Crit Rev Oncol Hematol 92, 153–165.
2 Lim, H. T. et al. (2022) Biom Eng Adv 4, 100054.
3 Harmsen, M. M. & De Haard, H. J. (2007) Appl Microbiol Biotech 77, 13–22.
4 Hurley, K. et al. (2023) Cancers (Basel)15, 3493.
5 Husain, B. & Ellerman, D. (2018) BioDrugs 2018 32:5 32, 441–464.
6 Medina Pérez, V. M. et al. (2024) Cancers (Basel) 16, 2681.
7 Fasciana, T. et al. (2021) Journal of Fungi 7, 551.
8 Jiang, Z. et al. (2023) Dis Markers 2023, 5178750.
9 Yu, Ting et al. (2024) Immunol Rev 328, 98.

Lead development: Llama/alpaca immunizations

Immunizations

Immunization strategies

For the generation of desired sdAbs, a strong immunization strategy is crucial.[1,2,3] QVQ can include one or a combination of several different antigens. These can be natural or recombinant proteins (e.g. His-tagged, Fc-tagged), peptides (e.g. conjugated to KLH, ovalbumin, albumin), and more complex samples such as cells and tissues. To obtain a wide variety of sdAbs, as a standard, two llamas are immunized with the same protocol. All immunizations are conducted according to the applicable animal welfare act in place and reviewed by the animal experiment commission of the company.

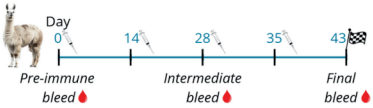


Figure 1. Standard immunization campaign.

Deliverables

- Design and implementation of an immunization protocol in llamas/alpacas
- RNA for library construction
- Serum samples
- Determined immune response

Immune response

During the immunization campaign, blood samples are taken from the llama/alpaca (Figure 1). Subsequently, the titers of antigen-specific antibodies in sera are determined by ELISA or flow cytometry (Figure 2).

Examples:

A

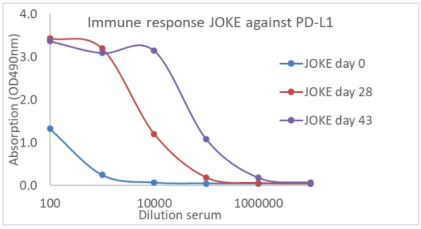


Figure 2. Example of Immune response of llamas against the immunization antigen.
A) Immune response against PD-L1 determined by ELISA.

References:
1 Van der Linden et al (2000) J Immunol Methods 240(1-2):185-95
2 Muyldermans et al (2008) Vet Immunol Immunopathol. 128(1-3):178-83
3 Arras et al (2023) Mabs 15(1):2261149

Lead development: VHH-phagemid libraries

VHH-phagemid libraries

Immune libraries

The RNA of immunized llamas is used to generate immune libraries. QVQ picks up VHH gene regions by PCR from reverse-transcribed RNA from PBMCs and clones the into the proprietary phagemid vector. In the phagemid vector, the VHH genes are fused to a C-terminal tag and the bacteriophage coat protein pIII. The high quality libraries provided by QVQ have an average size of ~10^8 different VHH.

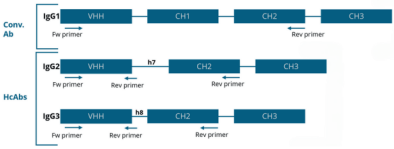
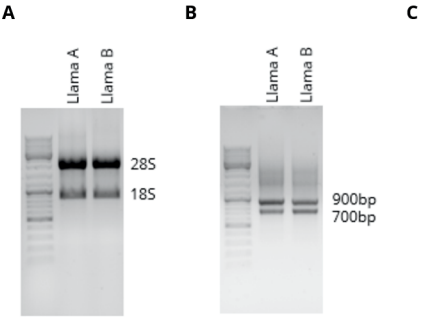


Figure 1. Primer annealing sites library construction

Deliverables

- Phage libraries with size ~10^8 transformants
- Report

Examples:



D

Llama	Immunized with	Tag	# of transformants	OD_{600}
Llama A	Antigen X	His	3E+08	96
Llama B	Antigen X	His	3E+08	95

Figure 2. Generation of the VHH-phagemid libraries Llama A and Llama B. A) Gel electrophoresis of 2 µl of the precipitated RNA samples on a 1% TBE agarose gel. The 28S and 18S ribosomal RNA bands are indicated. B) Amplification of the VHH (~700bp) and the VH (~900bp) cDNA, analyzed on a 1% TBE agarose gel. C) 10-fold dilutions of TG1 *E. coli* cells transformed with the VHH libraries, spotted onto LB-agar plates supplemented with 100 µg/ml ampicillin and 2% glucose. D) Summary table of the generated libraries.

Lead development: Ready-to-use libraries

Ready-to-use libraries

QPoLi
- **Pooled**
- >100 animals
- ~10^{11} transformants

QSyLi
- **Synthetic**
- Based on >1500 unique, good-producing sdAbs
- Theoretical diversity 10^{30}

QPoLi - the large pooled sdAb library

To allow immediate panning without immunizations, QVQ has generated a large, pooled library (QPoLi) from >100 different immune libraries that contains ~10^{11} sdAb-phagemid transformants (Figure 1). QVQ has dedicated protocols for working with libraries with large diversity.

QSyLi - a VH3-3-based synthetic sdAb library

Based on over a decade of sdAb sequence experience, QVQ also designed a synthetic library (QSyLi) that resembles the most prominent VHH germline (VH3-3) with germline-matched diversifications of both frameworks and CDRs. This germline is characterized by a long, kinked CDR3, which results in a relatively large paratope and good binding affinities (Figure 2). In addition, QVQ has incorporated protein A binding motifs in the frameworks to facilitate large-scale purification of lead clones.

Synthetic libraries can also be designed and generated for other sdAb germlines. QVQ can custom-design and generate synthetic variant phagemid libraries for your sdAb of choice.

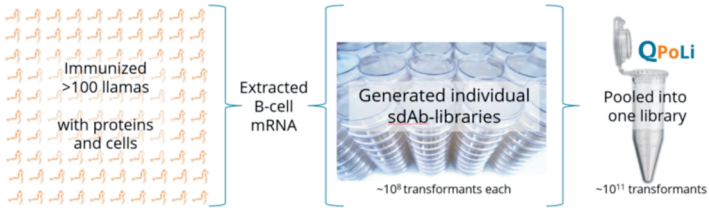


Figure 1. Construction of library QPoLi

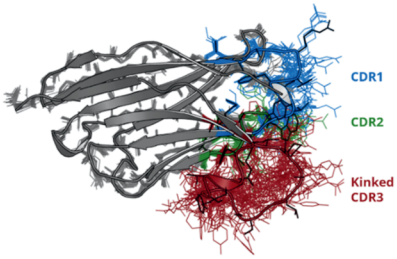


Figure 2. Overlay of structures of 20 synthetic sdAb from the QSyLi library.

Lead development:
Customized phage-display biopanning

Phage-display biopanning

QVQ always proposes a phage-display selection strategy tailored for the selection of the desired target binders. Such a strategy is carefully designed to include customer's requests and wishes. Importantly, the prospective final application of the single-domain antibodies is already taken into consideration while determining the phage-display selection strategy. The panning can be tailored to select for, e.g. agonistic or antagonistic single-domain antibodies, targeting preferred epitopes, high stability, or high specificity.

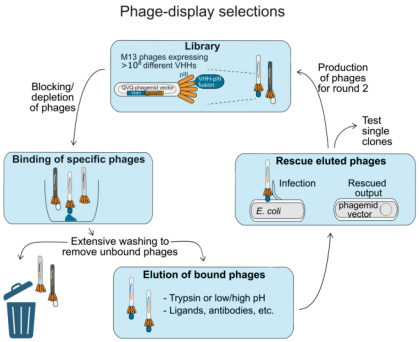


Figure 1. The generation of antigen-specific sdAbs by biopanning.

Biopanning

QVQ performs biopanning to select sdAbs specific to the target of interest out of the vast number of sdAbs in the immune or non-immune libraries. Phages expressing a sdAb, are allowed to bind antigen, e.g. recombinant protein, membrane extracts or cells. After removing the unbound fraction of phages, bound phages are eluted and rescued by infecting *E. coli*. These outputs, enriched with binders against the antigen, can be used to produce new phages for subsequent rounds of panning (Figure 1).

Due to the unique format of sdAbs and QVQ's optimized procedures, 2 rounds of panning is usually sufficient to enrich libraries for binders with high affinity. This process can be customized by applying counterselection (subjecting phages to antigen not desired as target, in solution), shielding undesired epitopes with e.g. ligands or antibodies, or subjecting the phages to specific conditions (e.g. low/high pH, specific elution).

Deliverables

- Outputs of the different libraries with different selection conditions

Examples:

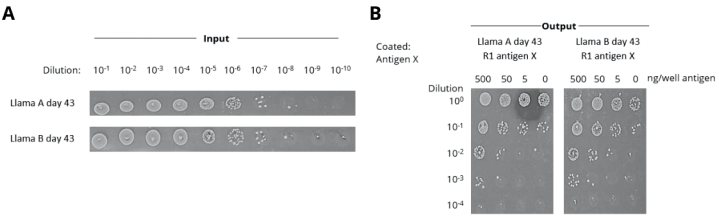


Figure 2. Example of panning against an antigen. A) Input phages were diluted in a range up to 10^{10}-fold and used to infect *E.coli* TG1 before spotting on selective LB agar plates. B) Results of the selection output on wells coated with indicated amount of directly coated antigen.

Lead development:
Screening hit picks for target binding

Screening

After phage-display biopanning, masterplates are made with 92 monoclonal sdAb each, representing the outcome of the panning efforts. Crude periplasmic extracts of the individual sdAbs are characterized for binding and other desired characteristics by ELISA and/or flow cytometry.

Deliverables

- Masterplate(s) with 92 monoclonal sdAb each, representing the outcome of the panning efforts
- Screening for binding to antigen in ELISA or flow cytometry

Examples:

A

XXX-1 Against Antigen X

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	1	2	2	3	3	4	4	5	5	6	6
B	1	1	2	2	3	3	4	4	5	5	6	6
C	1	1	2	2	3	3	4	4	5	5	6	6
D	1	1	2	2	3	3	4	4	5	5	6	6
E	1	1	2	2	3	3	4	4	5	5	6	6
F	1	1	2	2	3	3	4	4	5	5	6	6
G	1	1	2	2	3	3	4	4	5	5	6	6
H	1	1	2	2	PER	EM	4	4	5	5	IRR	EM

		Round 1				Round 2			
#	Library	Antigen	ng/well	Elution	Note	Antigen	ng/well	Elution	Note
1	Llama 1	Antigen X	500	Elution	TEA	Antigen X	500	Elution	TEA
2	Llama 1	Antigen X	50	Elution	TEA	Antigen X	50	Elution	TEA
3	Llama 1	Antigen X	5	Elution	TEA	Antigen X	5	Elution	TEA
4	Llama 2	Antigen X	500	Elution	TEA	Antigen X	500	Elution	TEA
5	Llama 2	Antigen X	50	Elution	TEA	Antigen X	50	Elution	TEA
6	Llama 2	Antigen X	5	Elution	TEA	Antigen X	5	Elution	TEA

B

Peri-screen by ELISA

XXX-1 Antigen X

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.84	0.87	1.46	1.19	1.12	1.10	0.69	0.46	1.63	0.05	0.05	0.05
B	1.36	1.85	1.54	1.49	1.44	1.32	0.65	0.28	0.05	0.06	0.55	0.05
C	2.16	2.34	1.67	1.63	1.60	1.50	1.36	1.57	1.54	0.06	0.61	2.62
D	1.41	1.87	1.72	1.84	1.80	1.68	0.05	1.65	2.76	1.65	1.73	0.06
E	1.82	1.37	1.70	1.76	1.78	1.51	1.69	1.40	0.05	1.36	0.05	0.05
F	0.50	0.05	1.81	1.84	1.75	0.74	1.41	2.11	1.02	0.05	0.05	0.06
G	2.92	1.75	1.72	1.69	1.67	1.05	0.05	1.33	1.88	0.05	0.05	0.09
H	2.38	1.47	1.68	1.63	0.05	0.05	2.31	0.98	1.18	0.06	0.05	0.05

C

Peri-screen by flow cytometry

Cell line expressing antigen of interest

	1	2	3	4	5	6	7	8	9	10	11	12
A	75635	136210	175234	175278	34246	156159	29735	47722	82539	106452	82958	155500
B	101034	103070	128091	162642	129531	5982	147017	70561	70431	110363	27065	16552
C	15908	3183	186942	149031	146047	37309	32725	25422	109498	81196	81869	95920
D	196042	154211	122941	752	128343	102667	33203	1431	20737	112506	92005	103354
E	125266	160104	120593	117789	153380	24796	90502	48020	88634	1163	98846	84560
F	6865	158249	82743	185998	101179	61750	14235	33718	88601	75575	97317	96925
G	131849	156448	143929	925	85516	25635	5066	5209	95445	78362	38672	93532
H	154674	9358	185086	153854	672	641	44699	14239	118643	96632	614	602

Background cell line

	1	2	3	4	5	6	7	8	9	10	11	12
A	583	587	609	611	603	600	596	586	593	606	607	614
B	584	572	587	599	595	616	593	596	606	600	602	616
C	572	586	598	606	597	602	598	616	616	621	595	608
D	576	580	587	597	591	602	598	588	599	600	597	598
E	583	587	605	599	603	612	605	627	626	617	606	620
F	601	599	590	608	612	614	609	626	635	639	614	606
G	601	588	599	581	604	596	622	645	605	607	605	601
H	593	595	593	595	587	601	614	621	606	588	609	560

Figure 1. Example of screening periplasmic extracts. A) Layout of masterplate XXX-1 containing single clones from the outputs of the selections. The following controls were taken along: empty phagemid vector (PER), irrelevant VHH in the phagemid vector (IRR) and empty (no bacteria) well (EM). The numbers indicated in the plate correspond to the selection output from which the monoclonal VHH originates and are shown in the table. B) Periplasmic extracts screened for binding to coated antigen (green) by ELISA. C) Periplasmic extracts screened for binding to cell-expressed antigen (blue) and its background cell line by flow cytometry.

Lead development:
Sequence analyses and hit picking

Sequence analyses

To determine the diversity of the VHHs, the entire masterplate will be sequenced by Sanger sequencing. The nucleic acid sequences will be analyzed and processed into VHH amino acid sequences using the PipeBio Antibody Sequence Analysis platform. Sequences will be correctly annotated for VHH framework and CDR sequences. Subsequently, the VHH sequences will be clustered by >80% CDR-H3 homology. QVQ will select a lead panel of at least 6 sdAbs based on a combination of binding data and sequence data.

Deliverables

- Sequence data
- Sequence analyses and annotation of clusters

Examples:

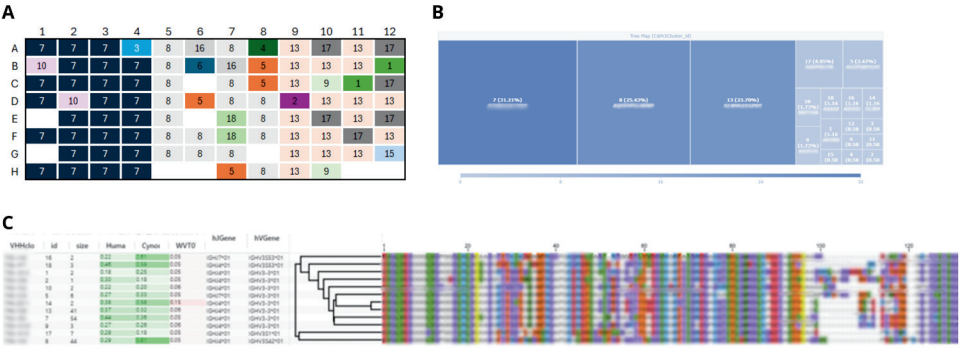


Figure 1. Example of sequence analysis. A) Masterplate layout in which the CDHR3 cluster ID numbers are indicated. B) Treemap indicating the relative size of the VHH clusters found. Cluster number and CDR-H3 sequence are depicted. C) Alignment of the amino acid sequences of the picked clones.

Lead development:
Validation of target binding of hits

Validation of target binding of hits

QVQ's standard quality control for purified sdAbs consists of UV-Vis spectroscopy and SDS-PAGE analysis for determination of protein concentration, integrity, and sample purity (Figure 1). Additionally, the composition of the native sample can be further analyzed by size-exclusion chromatography (SEC).

Antigen binding and estimated apparent binding affinities of purified sdAbs are routinely determined by ELISA. QVQ also offers binding analyses on cells by cell-based ELISA or flow cytometry using an Attune Nxt (Figure 2).

Deliverables

- At least 0.2mg of purified VHH protein (>90% pure, from *E. coli*) of a panel lead clones representing the sequence clusters
- cDNA of the purified clones in standard *E. coli* expression vector
- Determined estimated apparent binding affinities

Examples:

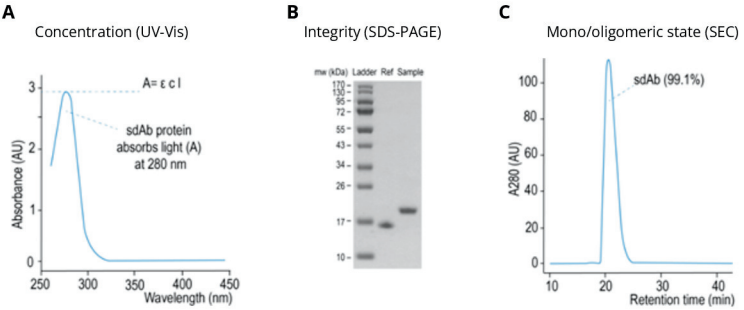


Figure 1. Quality control of purified sdAb. A) Absorbance at 280 nm. B) SDS-PAGE of sdAb sample (far right) compared to a (tag less) reference sdAb and a marker. C) SEC analysis of a sdAb.

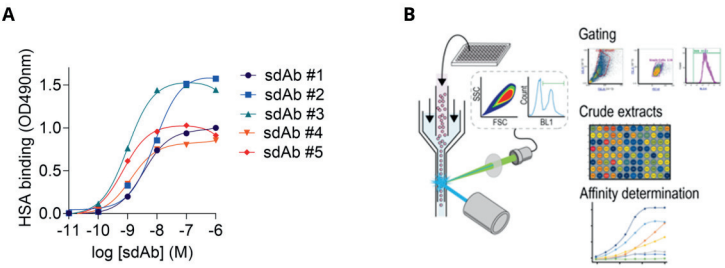


Figure 2. Determination of estimated apparent binding affinities. A) Binding curve determined by (cell-based) ELISA. B) Procedure of determining a binding curve by using flow cytometry.

Antibody discovery: Basic project

Consultation	- Project plan - Agreement - Work order
Antigens	- ~1 mg antigen protein - QC data
Immunization	- Immunization schedule → 2 llamas - 4 injections, 3 bleeds - Pre- and post immunization sera and RNA (PBMC) - Serum titration ELISA data
Immune libraries	- 2x VHH-phagemid libraries, *E. coli* glycerol stocks - QC data (RNA, PCR products, transformation)
Biopanning	- 2-3 rounds, 2 libraries - Phage titration data (input and output) - 2 masterplates with 92 monoclonal VHH
Screening	- Screening ELISA - Analyzed sdAb sequences, annotated and clustered - List of 6-12 hit picks for further characterization
Hit validation	- 0.2 mg of purified protein of 6-12 different sdAbs - Apparent binding affinity data in ELISA
Final deliveries	- All identified sequences - cDNA and protein of hits - All data and all IP - Project report

Year: 2025
Duration: 20 weeks
Costs: €38K

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Antibody discovery: Extensive project

Consultation	- Project plan - Master Service Agreement - Work order
Antigens	- hu/mouse/cyno recombinant protein + QC - Peptide-KLH - Target-expressed cells
Biopanning ready-to-use libraries	- Use of 2 non-immune libraries: 1) QPoLi: natural pooled VHH library 2) QSyLi: cloning of synthetic VHH library - 2 round of biopanning
Immunization	- Immunization schedule → 2 campaigns, 4 llamas 1) 4x rec. prot, 3 bleeds 2) 4x pept. + 2x boost (cells), 4 bleeds - Pre- and post immunization sera and RNA (PBMC) - Serum titration ELISA data
VHH libraries	- 4 Immune VHH-phagemid libraries - QC data
Biopanning	- 2 rounds, 4 libraries - Panning on captured antigen & cells - Counter selection and specific elution - Phage titrations - 6 masterplates with 92 monoclonal VHH
Screening + NGS	- Screening ELISA - Analyzed MP sequences - NGS amplicons, 20 outputs, ~50k seqs/output - Sequence analyses - 24 hit clones
Hit validation	- 0.2 mg of purified protein of 24 different sdAbs - Apparent binding affinities (ELISA) - Apparent binding affinities (flow cytometry)
Final deliveries	- All identified sequences - cDNA and protein of hits - All data and all IP - Project report

Year: 2025
Duration: 36 weeks
Costs: €100K

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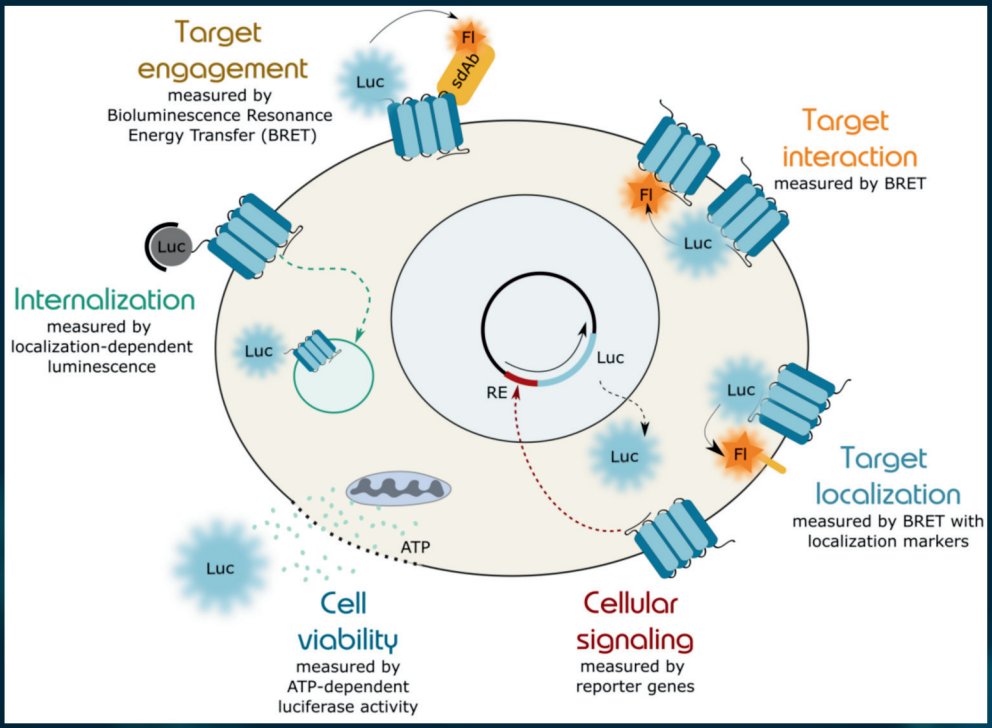
Optional follow up service: sdAb pharmacology

QVQ offers the following cell-based characterizations of sdAb pharmacology.

- **Target pharmacology**
  - Target engagement
  - Protein-protein interactions (target dimerization, target-effector interaction)
  - Target localization
  - Target internalization

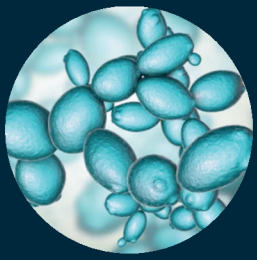
- **Cellular Responses**
  - Cell viability
  - Cellular Signaling (AP1, cAMP, NF-κB, NFAT, ERK/MAPK, G12/RhoA, Wnt/β-catenin)
  - Immune cell activation (ADCC, ADCP)



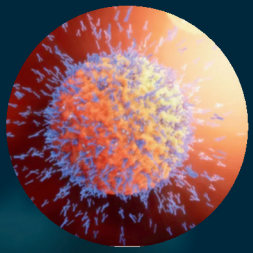
These can be applied to measure functional effects of purified sdAbs to evaluate agonism and antagonism as well as potencies and efficacies. Some of these can also be applied to crude periplasmic extracts for screening purposes.

Production of sdAbs or fusion proteins from different hosts and at various scales

Bacteria



Yeasts



Mammalian cells

Customized productions

- Tag of choice: with or without extra cysteine for conjugation
- Genetic fusions: multivalents, Fc-domains, enzymes, and more
- Affinity-purified
- Up to 1 gram
- Quality control: integrity, purity, affinity, endotoxin level

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QUALITY IN ANTIBODIES

Production of sdAbs or fusion proteins from different hosts and at various scales

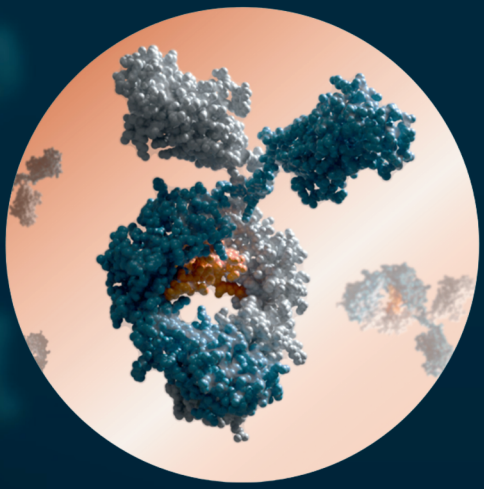
QVQ routinely produces sdAbs as recombinant proteins in ***E. coli***. For this purpose, sdAbs are equipped with a leader sequence that directs them to the bacterial periplasm and is cleaved off in the process. From the periplasm, the sdAbs are then purified via a C-terminal 6xHis-tag using immobilized metal affinity chromatography (IMAC).

To increase production yields or to incorporate an extra cysteine for conjugation, sdAbs can also be produced in **yeast** (*Saccharomyces cerevisiae*). Here, the sdAbs are equipped with a signal peptide causing them to be secreted from the cells.

For some proteins, like sdAb-containing fusion constructs, expression in **mammalian cells** can be beneficial. For instance, sdAbs fused to Fc domains of IgG antibodies or multivalent sdAbs can be expressed in e.g. HEK293 or CHO-K1 cells with high yields. Furthermore, expression in mammalian cells can optimize the folding and post-translational modification of the expressed proteins.

In case of production by yeast or mammalian cells, the sdAbs can be purified from the culture medium using affinity chromatography. Approximately half of all sdAbs can be purified via **protein A** chromatography. The other half can be either purified via an EPEA sequence in our proprietary C-Direct tag or can be engineered to incorporate protein A binding affinity. sdAb-Fc fusion molecules are also purified by protein A affinity chromatography.

After purification, all sdAbs are buffer-exchanged and quality controlled. sdAbs are provided in PBS unless requested otherwise.



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sdAb production in *E. coli*

Production in *E. coli*, purification from periplasm

Background

Escherichia coli (*E. coli*) is a well-established and efficient host system for the production of recombinant single-domain antibodies, offering rapid growth, low-cost cultivation, and a compatible environment for proper folding[1]. At QVQ, we routinely employ a *E. coli* BL21 strain[2].

Our sdAbs are equipped with a cleavable N-terminal leader sequence (pelB) that directs them to the bacterial periplasm, where the oxidative environment allows for proper folding and disulfide bond formation[3] (Figure 1). After expression, cells are lysed via a freeze-thaw cycle to release periplasmic contents, enabling access to the produced proteins. The sdAbs are purified using immobilized metal affinity chromatography (IMAC) via a C-terminal 6×His-tag. This production route is ideal for rapid screening and small-scale production relatively of good purity.

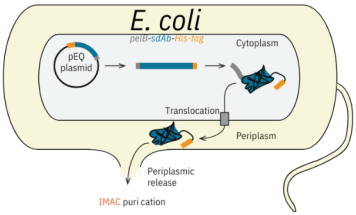

Figure 1. Schematic of sdAb production in *E. coli*. Single-domain antibodies (sdAbs) are expressed with a pelB leader sequence and C-terminal His-tag, guiding them to the periplasm where the leader is cleaved. Correctly folded sdAbs are released via cell lysis and purified via immobilized metal affinity chromatography (IMAC).

Service

SdAbs are produced in *E. coli* BL21 with yields up to 10 mg. SdAbs are standardly produced with His-tag or with FLAG-, V5- and Myc-tag as additional options. Our standard quality control includes UV-Vis spectroscopy, SDS-PAGE and ELISA to confirm antigen binding. Optionally, size-exclusion chromatography (SEC) can be performed to assess aggregation and homogeneity. Flow cytometry and SPR are available as additional services for binding assessment.

Deliverables

- Up to 10 milligram purified sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein characteristics (molecular weight, absorption coefficients, concentration)
  - Assessment of protein integrity (SDS PAGE, PageBlue stained; SEC analysis optional)
  - Apparent binding affinity to recombinant antigen (ELISA and optionally flow cytometry and SPR)

Examples:

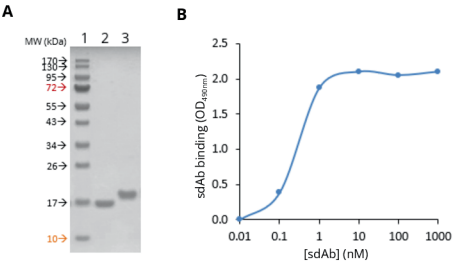

Figure 2. Quality control of QVQ product produced in *E. coli* BL21. A) SDS-PAGE of sdAb sample (lane 3) compared to a (tag less) reference sdAb (lane 2) and a marker. B) sdAb binding to immobilized recombinant protein in ELISA using OPD as substrate.

References:
1 de Marco (2020) Protein Expr. Purif. 172, 105645.
2 Huleani et al. (2021) Crit. Rev. Biotechnol. 42, 756–773
3 Kochendoerfer et al. (2007) Bioconjug. Chem. 18, 1897–1906

sdAb production in *S. cerevisiae*

Production in *S. cerevisiae*, purification from supernatant

Background

The yeast *Saccharomyces cerevisiae (S. cerevisae)* is a widely used eukaryotic host for recombinant protein production.[1] Its GRAS classification and endotoxin-free profile make it a safe and efficient system for recombinant sdAb production.[2] The produced sdAbs are secreted into the culture medium as a consequence of a SUC2 signal sequence.[3] In contrast to intracellular accumulation, sdAb secretion allows for high yields from either shake-flask production or batch-fed fermentation and simplyfies downstream processing. Moreover, *S. cerevisiae* copes well with the addition of an unpaired cysteine to the C-terminus of sdAbs for site-specific conjugation.[4]

Service

SdAbs are produced in *S. cerevisae* with yields up to 1 gram. Affinity chromatography is used to purify secreted sdAbs directly from the culture supernatant. Depending on the construct, purification is generally done via either Protein A or an EPEA-tag using CaptureSelect resin. Our standard quality control includes UV-Vis spectroscopy, SDS-PAGE and ELISA to confirm antigen binding. Size-exclusion chromatography (SEC) is performed to assess aggregation and homogeneity. Endotoxin levels can be determined upon request. Optionally, binding of the sdAbs can be assessed by flow cytometry and SPR.

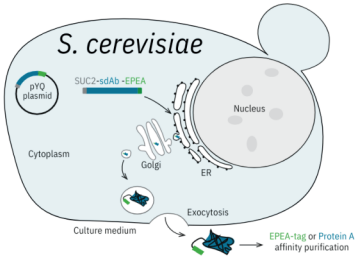

Figure 1. Schematic of sdAb production in *S. cerevisae*. sdAbs are expressed with a SUC2-secretion signal that directs them to the culture medium. Secreted proteins are harvested directly from the culture supernatant and purified by affinity chromatography using either intrinsic protein A-binding affinity or the attached EPEA-tag.

Deliverables

- Up to 1 gram purified sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein characteristics (molecular weight, absorption coefficients, concentration)
  - Assessment of protein integrity (SDS PAGE, PageBlue stained; SEC analysis)
  - Apparent binding affinity to recombinant antigen (ELISA and optionally flow cytometry and SPR)

Examples:

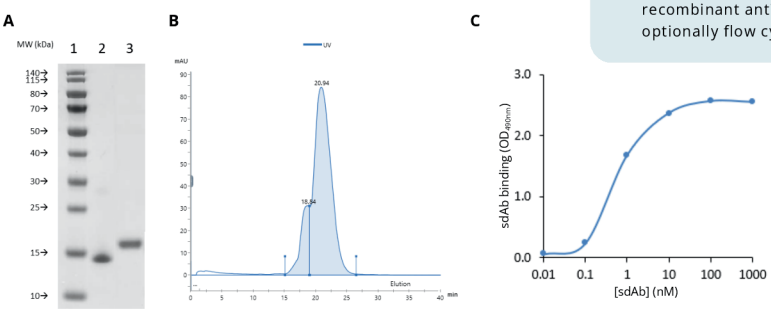

Figure 2. Quality control data of QVQ product, produced in *S. cerevisea*. A) SDS-PAGE of sdAb sample (lane 3) compared to a (tag less) reference sdAb (lane 2) and a marker B) SEC analysis showing 82% monomers and 18% cystine-mediated sdAb-dimers and 100% purity. C) Binding of Q17c to immobilized recombinant human HER2 in ELISA using OPD as substrate.

References:

1 Valenzuela P, Arch Biol Med Exp. 1988 Jun;21(1):231-40
2 Frenken LG, et al., J Biotechnol. 2000 Feb 28;78(1):11-21
3 Weinhandl K et al., Microb Cell Fact. 2014 Jan 9;13:5
4 Heukers R et al., Antibodies (Basel). 2019 Apr 4;8(2):26

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sdAb production in mammalian cells

Production of single-domain antibodies in mammalian cells

Background

Mammalian expression systems, such as HEK293 cells, are ideal for the production of Fc-tagged sdAbs when native folding, glycosylation, and other complex post-translational modifications are essential[1]. These systems are commonly used for applications that require enhanced stability or Fc-mediated effector functions. Moreover, its endotoxin-free profile makes it well-suited for sensitive applications, including therapeutic development[2].

At QVQ, codon-optimized sdAb-Fc constructs are expressed with a signal peptide (IgH leader) that directs secretion via the ER–Golgi pathway. This results in proper folding and assembly, followed by exocytosis into the culture medium. The secreted proteins are then purified from the supernatant using affinity chromatography via the Fc region binding protein A/G (Figure 1). This approach supports functional expression of full-length fusion formats and enables efficient, scalable purification from animal component-free media.

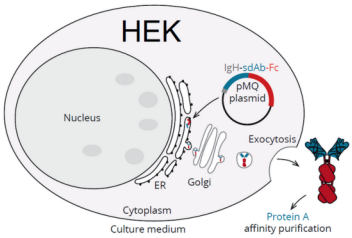


Figure 1. Schematic of sdAb-Fc production in HEK. sdAb-Fc fusion proteins are expressed with a IgH signal peptide that drives secretion into the culture medium. Secreted proteins are harvested directly from the culture supernatant and purified via affinity chromatography using the Fc domain's high affinity for Protein A.

Service

SdAbs are produced in Expi293- or CHO-K1 cells with yields up to 1 gram. Our standard quality control includes UV-Vis spectroscopy, SDS-PAGE and ELISA to confirm antigen binding. Size-exclusion chromatography (SEC) is performed to assess aggregation and homogeneity. Optionally, binding of the sdAbs can be assessed by flow cytometry and SPR.

Deliverables

- Up to 1 gram purified sdAb in PBS
- Certificate of Analysis (CoA) containing:
  - Protein characteristics (molecular weight, absorption coefficients, concentration)
  - Assessment of protein integrity (SDS PAGE, PageBlue stained; SEC analysis)
  - Apparent binding affinity to recombinant antigen (ELISA and optionally flow cytometry and SPR)

Examples:

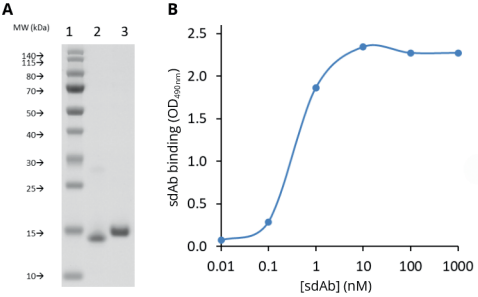


Figure 2. Quality control of QVQ product produced in ExpiHEK 293 cells. A) SDS-PAGE of sdAb sample (lane 3) compared to a (tag less) reference sdAb (lane 2) and a marker. B) Binding of sdAb to immobilized recombinant protein in ELISA using OPD as substrate.

References:
1 Jäger et al. (2013) Cell. Mol. Life Sci. 70, 603–618.
2 Zhang et al. (2024) Biochem. Eng. J. 205, 109279.

Off-the-shelf reagents

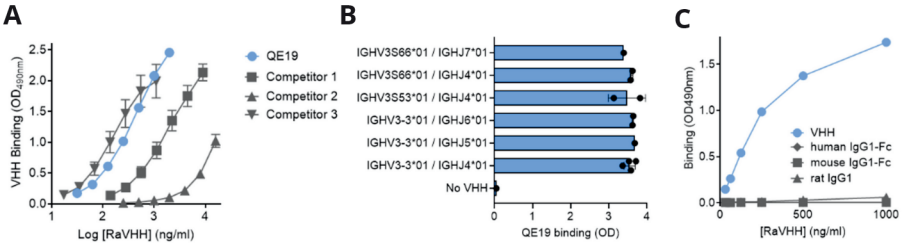
Target	sdAb
ACKR3	Q123, Q125, Q126c
B7-H3	Q92c
BMP2/4	Q36c
BMP4	Q35bc
CAIX	Q29c
Candidalysin	Q99c, Q100c
C3, C3b	Q122c, Q119c
C5	Q101c, Q102c
C5b6	Q120c, Q127c
CCR2	Q124c
CD63	Q111, Q112
CD80	Q98c
CD163	Q68c
Complement factor B	Q121c
CXCR4	Q84c, Q85c
DC-SIGN	Q51c, Q94c
EGFR	Q44c, Q86c
EpCAM	Q66c
Fc-domain	Q96c
Fibrinogen	Q113

Target	sdAb
Glycoprotein 1b α	Q114
Glycoprotein VI	Q115, Q116
HER2	Q17c
HIV gp120	Q1c
HIV gp41	Q8c, Q54c
HIV p24	Q76bc
IRDye800	Q93c
ITGB1	Q75c
cMet	Q22c
NCAM1	Q55c
PD-L2	Q90c
P-Selectin	Q117
SARS-CoV-2	Q103c, Q104c
TfR	Q52c
Tubulin	128c
uPAR	Q88c
Vimentin	Q60c
VLA3	Q48c
vWF	Q118

Rabbit-anti-VHH

Building on our expertise of generating and characterizing single-domain antibodies, QVQ has developed a unique antibody against llama single-domain antibodies.

QE19 is a polyclonal protein A-purified rabbit antibody with little to no cross-reactivity to human, rat and mouse IgG1. QE19 recognizes all VHH germlines and is suitable for ELISA, immunofluorescence, and flow cytometry.



Detection of VHH by QE19. A) Detection of VHH by QE19 and three different competitor rabbit-anti-VHH. B) Detection of VHH from different germline families by QE19 (1:5000 dilution). C) Detection of Fc domains from human, murine or rat IgG1 by QE19. Bound antibodies were detected using donkey-anti-rabbit-HRP and OPD as substrate.

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Contact us for the lead development and/or production of your sdAb!

info@qvquality.com

QVQ moved to a new state-of-the-art laboratory on the 12th floor of the Plus Ultra Utrecht building.

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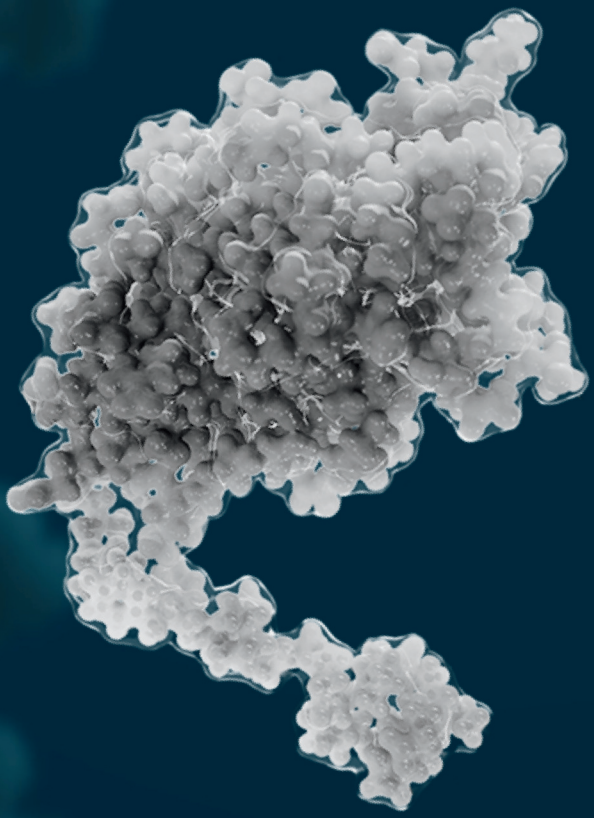

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