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# Technical information deep dive for Abcalis services, products and formats

## Services:

Every specific service is designed individually with and for the customer. The work packages described below represent a guideline for what certain projects at Abcalis GmbH commonly comprise.

Antibody generation (this is our core service)

- Aim: discovery of new antibodies against a target molecule of choice
- Antigens are provided by the client, acquired commercially, produced in house or in case of peptides synthesized by a third party
- Antibodies binding the antigen are selected from naïve libraries (diversity of 1.5 x $10^{10}$ different antibodies) via phage display
- Promising candidates are cloned into full IgG format of a host species of choice (usually human, mouse, rabbit or goat)
- Full IgG antibodies are produced and validated in ELISA
- Deliverables for customers:
    - IgG protein for internal R&D (up to a few mg; more (up to grams) are possible in a larger time frame due to existing partnerships with ISO certified contract manufacturers)
- Time frame: 10-16 weeks depending on project complexity
- For commercialization purposes and commercial use in general:
    - Full ownership of antibody sequences (acquirable through larger one-time fee)
    - Exclusive and non-exclusive licensing of antibody sequences (annual fee or net sales based royalty)

Antibody format conversion

- Aim: transformation of an antibody from one host species into another, while preserving binding characteristics
- (Optional): The customer provides the antibody sequence (at least VH and VL)
- DNA encoding the antibody is either also provided by the customer or acquired via gene synthesis
- Affinity defining VH and VL regions are cloned into one of the many formats available at Abcalis GmbH (usually full IgG from human, mouse, rabbit, or goat)
- Full IgG antibodies are produced and validated in ELISA
- Time frame: 2-4 weeks (gene synthesis depends on external providers)
- If the customer provided the antibody sequence, full ownership stays with customer, only a one-time service price applies.
- For antibodies converted which were previously under ownership of Abcalis, the following applies for commercialization purposes and commercial use in general:
    - Full ownership of antibody sequences (acquirable through larger one-time fee)

- Exclusive and non-exclusive licensing of antibody sequences (annual fee or net sales-based royalty).

Affinity maturation

- Aim: improvement of binding characteristics and affinity of a pre-existing antibody
- The customer provides the antibody sequence (at least VH and VL)
- DNA encoding the antibody is either also provided by the customer or acquired via gene synthesis
- Antigens are provided by the client, acquired commercially, produced in house or in case of peptides synthesized by a third party
- VH and VL sequences are randomized either in-house or via gene synthesis
- VH and VL sequences are cloned into phagemid vectors and packaged into a new antibody phage library
- From the new libraries antibodies binding the antigen are selected via phage display
- Promising candidates are cloned into full IgG format of a host species of choice (usually human, mouse, rabbit or goat)
- Full IgG antibodies are produced and validated in ELISA
- Deliverables for customers:
  - IgG protein (up to a few mg; more (up to grams) are possible in a larger time frame due to existing partnerships with ISO certified contract manufacturers)
- If the customer provided the antibody sequence, full ownership stays with customer, only a one-time service price applies.
- For antibodies converted which were previously under ownership of Abcalis, the following applies for commercialization purposes and commercial use in general:
  - Full ownership of antibody sequences (acquirable through larger one-time fee)
  - Exclusive and non-exclusive licensing of antibody sequences (annual fee or net sales-based royalty)
- Time frame: 10-16 weeks

Naïve library generation

- Aim: construction of antibody libraries from non-immunized donors → representation of the complete resting state antibody repertoire
- Blood, Buffy-coat, PBMC or B cell samples are procured from voluntary donors or acquired commercially
- Antibody VH and VL genes are amplified from the B cell fraction and cloned into phagemid vectors
  - Natural pairing of VH and VL is broken intentionally
  - Every VH is recombined with every VL to increase library diversity ("chain shuffling")
- Libraries are stored in large batches of glycerol stocks for long term use over many years
- Libraries are packaged into phage particles for direct use in phage display panning
- Possible deliverables for customers:
  - Library glycerol stocks
  - Ready-to-use packaged phage library
- Time frame: 4-6 months depending on the number of donors
- Service package price depends on the possibility for Abcalis to also have the rights to use and commercialize upon the generated library.

Immune library generation

- Aim: construction of antibody libraries from immunized animals → representation of the complete antigen activated antibody repertoire
    - If library generation is successful, no animal has to be sacrificed for the purpose of gaining antibodies against the antigen in question ever again!
- Blood or spleen samples are procured from immunized animals or preferably directly provided by the customer
- Antibody VH and VL genes are amplified from the B cell fraction and cloned into phagemid vectors
    - Natural pairing of VH and VL is broken intentionally
    - Every VH is recombined with every VL to increase library diversity ("chain shuffling")
- Libraries are stored in large batches of glycerol stocks for long term use over many years
- Libraries are packaged into phage particles for direct use in phage display panning
- Possible deliverables for customers:
    - Library glycerol stocks
    - Ready-to-use packaged phage library
- Time frame: 2-4 months depending on the number of animals used
- Service package price depends on the possibility for Abcalis to also have the rights to use and commercialize upon the generated library.

## Products:

- Multiclonals (defined mixes of recombinant antibodies that resemble polyclonals)
    - Anti-human IgG
    - Anti-rabbit IgG (end-stage development)
- Monoclonals ("classic" antibodies)
    - Anti-HA-tag antibody
    - Anti-human IgA antibody (under development)
    - Anti-human IgG4 antibody (under development)
    - "Hypermyc" anti-cmyc-tag antibody
    - anti-SARS-CoV-2 antibodies
- Sandwich pairs
    - anti-SARS-CoV-2 S1-protein sandwich pair
    - anti-SARS-CoV-2 N-protein sandwich pair
    - anti-CRP sandwich pair

## Formats:

Beside the usual host species (human, mouse, rabbit, goat), Abcalis develops and offers special antibody formats for different applications. Every Abcalis antibody can be acquired in every available format due to our recombinant technology. These formats can also be made available for customer antibodies via our format conversion service.

- TWIN-Strep tag: antibodies that carry a TWIN-strep tag are compatible with the Strep-tactin purification and detection system by iba technologies

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- Free-Cysteine: antibodes are engineered to have a free cysteine in a defined position for single covalent conjugation in a defined position
- His-tag: antibodies carrying a 6x histidine tag are compatible with all his-tag based purification and immobilization systems
- CBM3a: antibodies that carry a cellulose binding domain can be immobilized on cellulose membranes that are biodegradable in contrast to the widely used nitro-cellulose membranes
- CBM77 (under development): antibodies that carry a nitro-cellulose binding domain can be immobilized on nitro-cellulose membranes in a directional manner
- HRP: HRP conjugation is a standard in-house process for our detection antibodies and can be applied to any other
- Biotin: all antibodies can also be supplied in a biotinylated form

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