

## Internalisation Assay Services

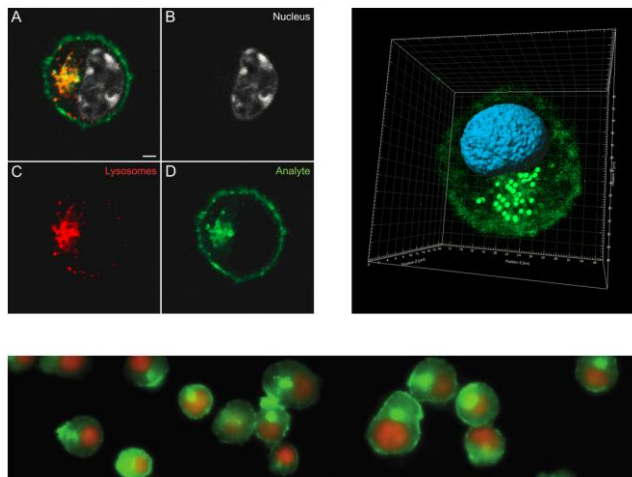
Today humanised monoclonal antibodies are considered highly innovative therapeutic tools in the field of cancer therapy and other fields indications.

With ever growing specificity of epitope recognition they hold great promise with regard to targeted and personalised therapies.

Benefits result from the specific addressing of diseased cells or tissues based on the expression of disease-relevant marker proteins.

Further developments regarding antibody drug conjugates (ADCs) open up the field to eventually substitute chemotherapeutic strategies, the latter often involving severe side effects.

Essential parameters like target/epitope specificity and cellular processing of bound antibodies ask for detailed analyses of therapeutic immunoglobulins which are to be transferred to clinical phases.



### Approach and Deliverables

#### Membrane association of primary antibodies

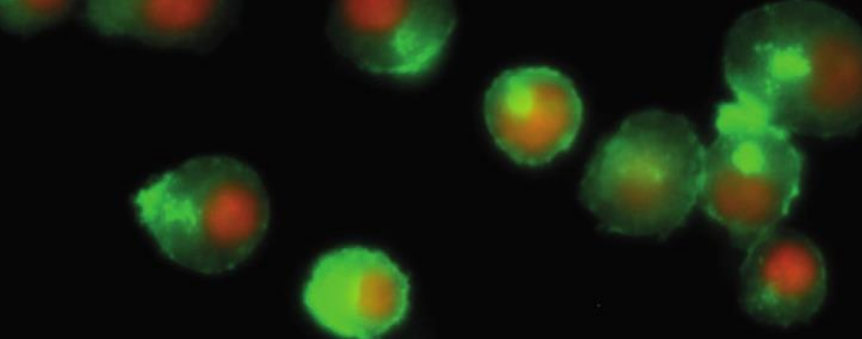
- Detection of fluorescently labeled antibodies via confocal microscopy
- Cellular segmentation via cytoplasmic membrane and nuclear staining
- Demonstration of membrane association of antibody via optical sectioning and/or 3D reconstruction of confocal Z-stacks

#### Time course of antibody internalisation

- Confocal documentation of fixed samples at defined times after initial antibody binding at high resolution in x, y, z dimension
- 2D-/3D time series of living samples after initial antibody binding
- Determination of intracellular (cytoplasmic) and extracellular (membrane) antibody localization over time based on cellular stains

#### Internalisation routes of antibodies

- Immunocytochemistry to label components of the endolysosomal pathway (i.e. early endosomes, lysosomes)
- Assessment of colocalisation of internalised antibody with endosomal and/or lysosomal markers via confocal microscopy
- Temporal profile of endosomal and/or lysosomal localization of internalised, intracellular antibody aggregates



## Internalisation Assay Services

### Installed imaging equipment

- Confocal laser scanning microscope Carl Zeiss LSM 510 META
- Spinning disc confocal live cell microscope Carl Zeiss Cell Observer SD
- Structured illumination confocal microscope Carl Zeiss Imager.Z1 Apotome
- Widefield fluorescence microscope Carl Zeiss Axiovert 200
- High End software solutions (Imaris, Bitplane AG; ZEN, Carl Zeiss Microscopy GmbH) for image processing and analysis

### Materials to be provided by customer

- Test samples of primary antibodies, fluorescently labeled if possible (~ 300 µg/antibody)
- Cell lines of choice in which antibody internalisation is to be evaluated (2 cryo vials/cell line; ~2 x 10<sup>6</sup> cells/cell line)

### Throughput & processing time

- Detailed analysis of internalisation properties of up to 5 antibodies in cell line of choice
- Submission of final service report within approx. 12 weeks after receipt of order
- Flexible arrangement of service packages to meet customers individual needs

