Immunoglobulin Fab Assay for Cedex Bio and Bio HT Analyzers

Reliable and convenient automated determination

Antigen-binding fragments of immunoglobulins (Ig Fab) are produced instead of complete IgG antibody molecules for special diagnostic and therapeutic applications.

The Ig Fab test on Cedex Bio and Cedex Bio HT Analyzers provides fast and accurate quantitative determination of human immunoglobulin Fab fragments (Ig Fab) in cell culture media and other aqueous solutions.

The immunoturbidimetric assay uses an antiserum which specifically binds to Ig Fab from the sample, and the evolving turbidity is measured photometrically.

Process control based on fast and reliable analytics

- High accuracy, results are consistent to HPLC
- No sample filtration or other pretreatment required
- Wide measuring range, option for on-board dilution
- Barcoded reagents, ready-to-use
- Calibration required only once per lot

Assay accuracy

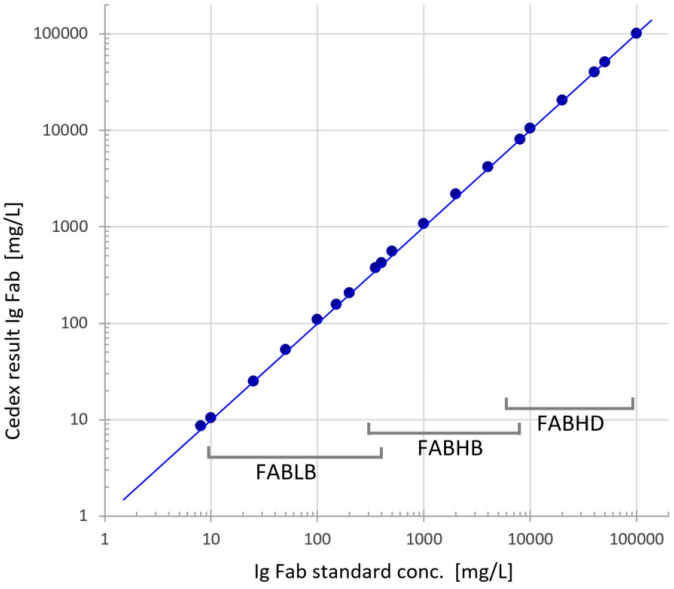

Fig. 1: Accuracy of Ig Fab determination

An Ig Fab standard was spiked into cell culture medium and determined on a Cedex Bio HT Analyzer. Using the three test protocols over the wide range of 8 – 100'000 mg/L, the recovery was always better than ±10%.
(Excerpt from verification data of Roche)

For use in quality control/manufacturing process only.

CUSTOMBIOTECH

Wide measuring range, low sample volume required

The same reagent is used with several protocols to cover a wide IgG concentration range.

Only 2 – 12 µl of sample are used, depending on the need for high sensitivity or automated predilution for high product concentrations.

Protocol	IgG range	Sample vol.
FABLB low range	10 - 400 mg/L	12 µL
FABHB high range	300 – 8 000 mg/L	10 µL
FABHD high + dilution	6 000 – 40 000 mg/L	2 µL

Assay principle

The turbidimetric immunoassay uses an antiserum with polyclonal detection antibodies directed against Ig Fab. The binding to Ig Fab from a sample generates an emerging turbidity. The absorbance at 340 nm relates to the concentration of Ig Fab in the sample. In a subsequent step, additional immunoglobulin is added to the reaction to verify that there is still excess of the detection antibody and the signal was not suppressed by excessive antigen (Ig Fab).

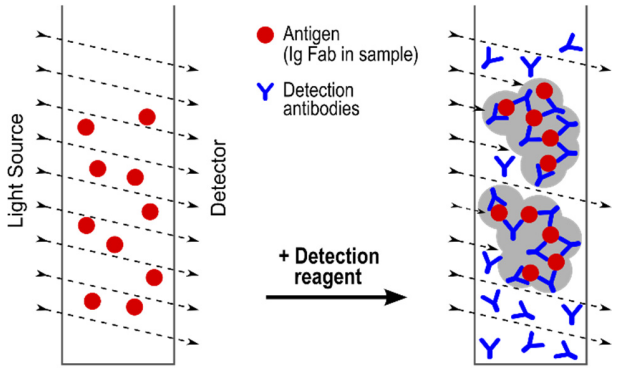

Fig. 2: Turbidimetric immunoassay
The divalent detection antibodies bind to Ig Fab from the sample. The light is scattered by emerging turbidity of the antibody-antigen complex and the intensity at the detector is reduced in dependence on the amount of target molecules.

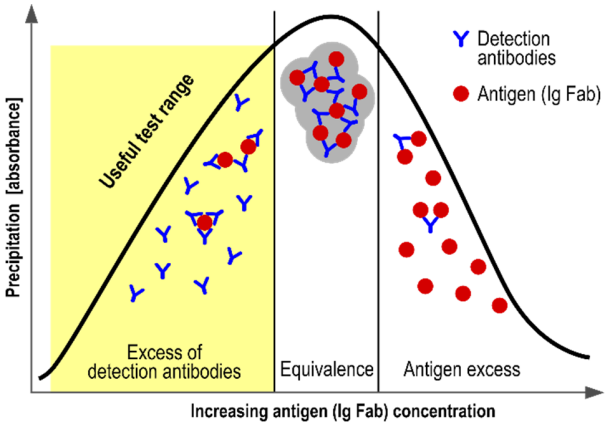

Fig. 3: Heidelberger curve
In the immunoturbidimetric assay with a constant concentration of detection antibodies, the precipitation increases with a rising amount of Ig Fab from the sample (= antigen). Equivalent concentrations of antibodies and antigen reach the maximum of turbidity, and with further rise of antigen the precipitation decreases. Therefore, the determination of the Ig Fab concentration is only feasible with an antibody excess, and an antigen excess has to be avoided.

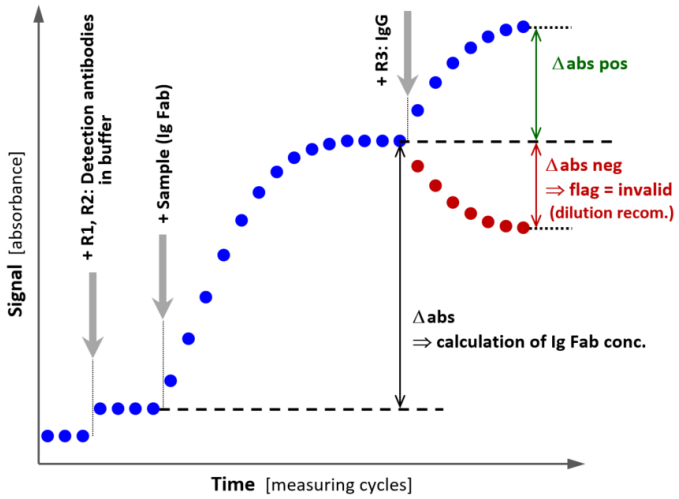

Fig. 4: Cedex test protocol for Ig Fab determination including verification step: After mixing of reaction buffer (R 1) and detection antibodies (R2), sample is added and the increase in absorption due to the evolving turbidity is measured for calculation of the Ig Fab concentration. Subsequently, additional IgG (R3) is added to check for antigen excess. Further increase of absorbance confirms the validity of the result. If the absorbance is constant or decreasing, the result will be flagged as invalid due to antigen excess (sample concentration much higher than test range) or due to interfering substances in the sample. Then the test should be repeated by selection of another Ig Fab protocol for a higher test range (lower amount of sample in the reaction).

Specificity

The goat antiserum in the assay specifically binds to epitopes in the constant region of kappa-type light chains of the Fab fragment of human immunoglobulins.

Using this assay, the following molecules can be determined:

- free light chains
- Fab fragments (papain digest of antibodies)
- F(ab')$_2$ fragments (pepsin digest of antibodies)
- full antibodies (IgG, IgM, IgE, …)
- immunoglobulin constructs for analytical or therapeutic use

For determination of complete immunoglobulin G antibodies (IgG), the Fc region of the molecule is the preferable assay target, because the large Fc region enables sensitive detection and its structure is highly conserved between different immunoglobulin molecules (refer to the Cedex IgG kit).

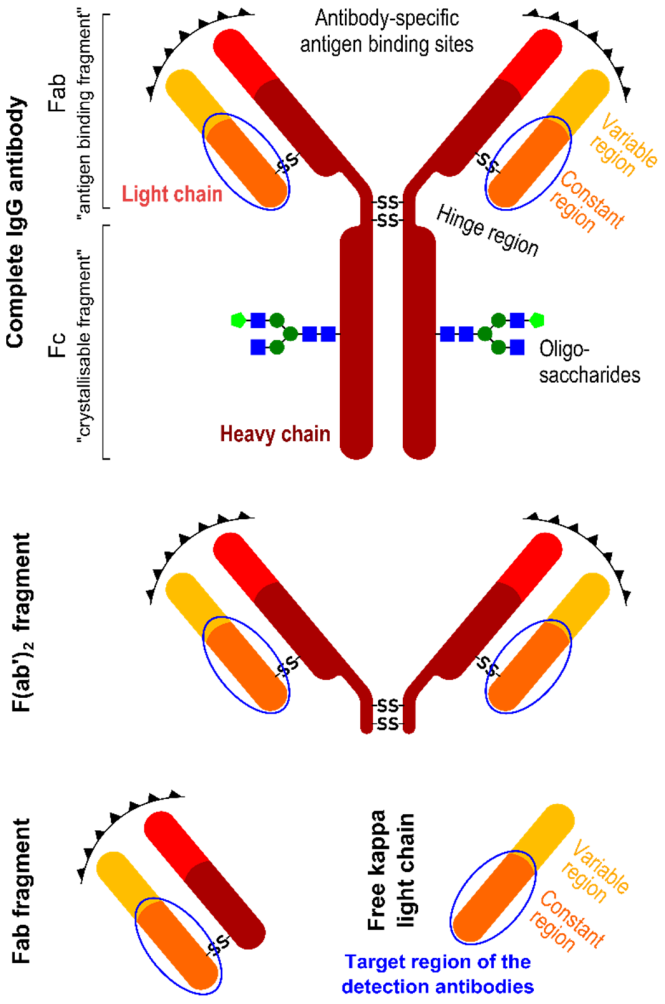

Fig. 5: Assay target molecules

The detection antibodies in the assay are directed against the constant region of human kappa light chains. All molecules containing this target structure can be determined in this test.

A growing number of artificially designed recombinant immunoglobulins for therapeutic use is reduced to the Fab fragment only, which

- binds to the antigen like a full antibody,
- does not interfere with cells of the immune system (function mediated by Fc receptors),
- is able to penetrate into tissue due to the smaller molecule size (about 1/3 of IgG).

Such molecules can be determined using the Ig Fab assay.

Optimal accuracy by specific calibration

The provided 'Calibrator A Bio' (CAL A) contains a typical IgG molecule of 150 kDa for generic calibration of the assay, however, the smaller Fab fragments and light chains differ in molecule size and signal intensity in the assay. Therefore, with a generic calibration using CAL A, the results of Fab testing will show a clear correlation to the actual concentrations, but the accuracy may be weak.

For optimal accuracy, we recommend to calibrate the assay with a standard solution of the specific molecule to be determined. The custom calibrator solution should have a concentration of about 300 mg/L of the specific protein (within 250 - 400 mg/L), exactly determined by a reference method like HPLC or absorbance at 280 nm. Controls for verification of the test accuracy should also be based on the same protein.

Additional protocol codes for the Cedex Analyzers with copies of the Ig Fab protocols are available on request, enabling the parallel use of several specific calibration curves for different molecules with the same reagent on the same instrument.

Ordering information

Product	Pack size	Catalog no.
Ig Fab Bio [1]	4 x 50 tests	08 881 332 001
Ig Fab Bio HT [1]	200 tests	08 881 359 001
Calibrator A Bio [2] (optional)	6 x 1 ml	06 682 189 001

Regulatory disclaimer

[1] For use in quality control/manufacturing process only.

[2] For quality control/manufacturing of IVD/medical devices/pharmaceutical products only.

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