Human IgG Assay for Cedex Bio and Bio HT Analyzers

Reliable and convenient automated determination

The IgG assay developed for Cedex Bio and Cedex Bio HT Analyzers provides fast and accurate quantitative determination of human immunoglobulin G (IgG) in cell culture and fermentation media.

The assay is based on an immunoturbidimetric method: A specific antiserum reacts with IgG from a sample and the evolving absorbance 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, on-board dilution capability
- Barcoded reagents, ready-to-use
- Calibration required only once per lot

Method comparison

IgG test results from Cedex Analyzers are consistent to established methods like HPLC with Protein A columns.

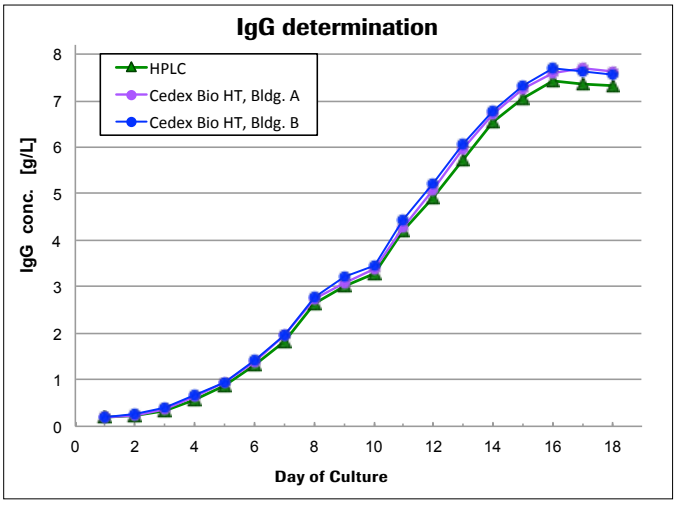

Fig. 1: Method comparison Cedex ⇔ HPLC
The IgG titer in a CHO cell culture was continuously monitored using HPLC (Protein A) and two independent Cedex Bio HT Analyzers in different labs. Results are equivalent, deviations < 3%.
(Excerpt from verification data of Roche Pharma)

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

Protocol	IgG range	Sample vol.
IGGLB low range	10 - 80 mg/L	25 µL
IGGHB high range	80 – 1600 mg/L	2 µL
IGGHD high + dilution	400 – 8000 mg/L, up to 160 g/L with higher dilution	40 µL

Assay principle

The turbidimetric immunoassay uses an antiserum from rabbit with a detection antibody against human immunoglobulin G (IgG). The binding of IgG from a sample to the detection antibody generates an emerging turbidity. The absorbance at 340 nm relates to the concentration of IgG in the sample. In a subsequent step, additional IgG is added to the reaction to confirm that there is still excess of the detection antibody and to verify that the signal was not suppressed by excessive antigen (IgG) or other interfering substances.

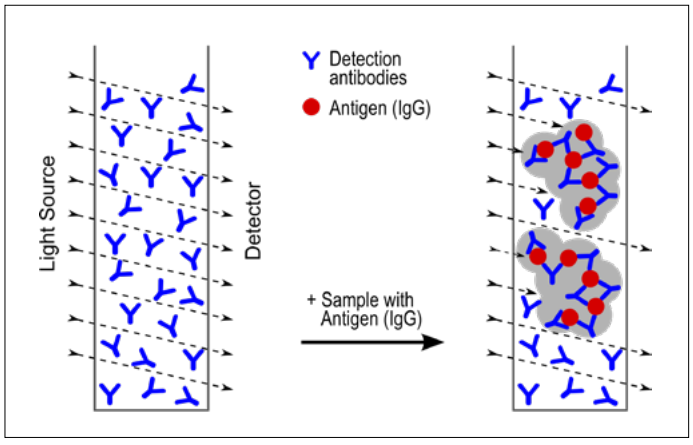

Fig. 2: Turbidimetric immunoassay
The detection antibody binds to IgG from the sample. The light is scattered by emerging turbidity of the antibody-antigen complex and the intensity at the detector is reduced in correlation to the amount of target molecules.

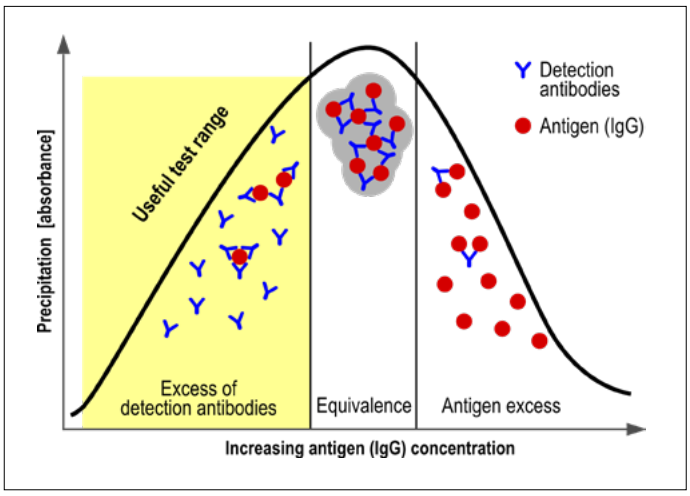

Fig. 3: Heidelberger curve
In the immunoturbidimetric assay with a constant concentration of detection antibodies, the precipitation increases with a rising amount of IgG from the sample (= antigen). Equivalent concentrations of antibodies and antigen reach the maximum of turbidity, and with further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

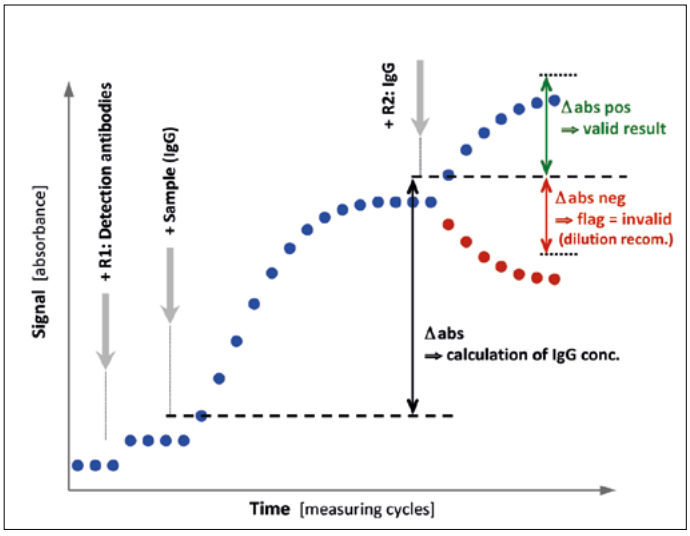

Fig. 4: Cedex test protocol for IgG determination with verification
After mixing of detection antibodies (R 1) and sample, the increase in absorption (turbidity by precipitation) is measured for calculation of the IgG concentration.

Subsequently, additional IgG (R 2) 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 (concentration of IgG higher than test range) or due to interfering substances in the sample. If the result is flagged, then the test Vshould be repeated by selection of another appropriate IgG protocol for a higher test range (lower amount of sample in the reaction).

Specificity

The rabbit antiserum in the assay is specific for the Fc part of human IgG molecules, which has a highly conserved structure important for effector cell activation in immune reactions *in vivo*. All subtypes of human IgG (IgG1, IgG2, IgG3, IgG4) can be detected using this kit. No signal will be observed with IgG from species other than human or other types of immunoglobulins (IgA, IgM, IgE).

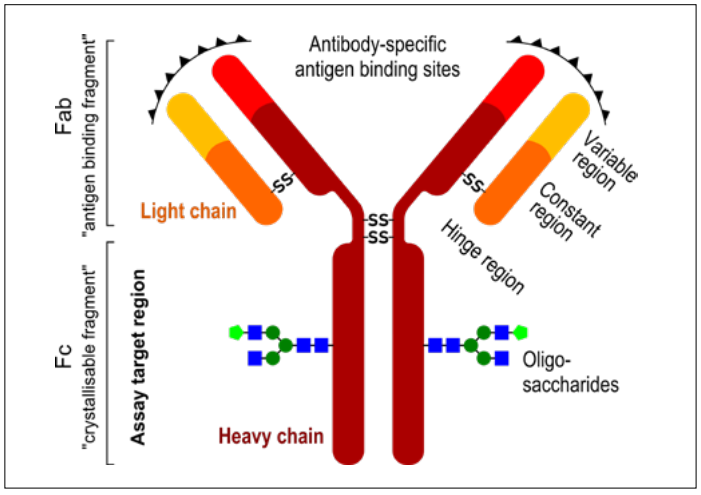

Fig. 5: The binding site for the detection antibodies of the IgG assay is the human IgG Fc fragment in the constant region of the heavy chains.

Optimal accuracy by specific calibration

The provided 'Calibrator A Bio' (CAL A) contains a common IgG1 molecule of 150 kDa for generic calibration of the assay, resulting in a good test accuracy (typically within 5%) for the majority of human IgG molecules.

Nevertheless, individual IgG products differ in molecular structure and size and therefore the binding affinity in the assay can be slightly different. This may cause a product-specific deviation from the generic calibration with up to 20% inaccuracy (in worst case), especially in the upper third of the measuring range.

Optimal accuracy over the whole range will be achieved in every case with a custom calibration using a standard solution of the specific IgG product instead of CAL A. The custom calibrator solution should have a concentration of about 1.5 g/L IgG (within 1.35 - 1.7 g/L), exactly determined by a reference method like Protein A HPLC or absorbance at 280 nm. Controls for verification of the test accuracy should also be based on the specific IgG product.

Additional Cedex Analyzer protocols are available. This enables the parallel use of several product-specific calibration curves with the same Cedex reagent on the same Cedex Analyzer.

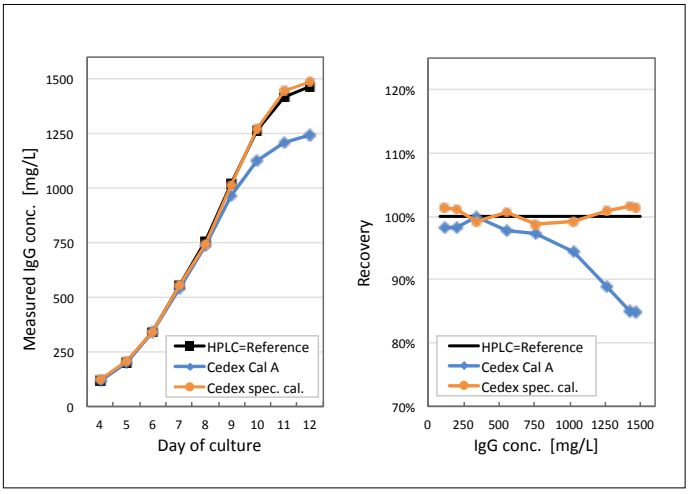

Fig. 6: Option for product-specific calibration
With some IgG products like shown in this experiment, there may be a deviation in the binding affinity compared to the generic calibrator "CAL A", especially in the higher part of the concentration range. With a custom calibration using a standard solution of the specific IgG product ("spec. cal."), optimal accuracy is always achieved over the whole range.
(Excerpt from verification data of Roche Pharma)

Ordering information

Product	Pack size	Catalog no.
IgG Bio	4 x 50 tests	06 681 743 001
IgG Bio HT	100 tests	06 608 540 001
Calibrator A Bio	6 x 1 ml	06 682 189 001
Control A Level 2 Bio	6 x 1 ml	06 682 227 001
Control A Level 3 Bio	6 x 1 ml	06 682 545 001

Regulatory disclaimer

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