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)
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.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>IgG range</th>
<th>Sample vol.</th>
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<tbody>
<tr>
<td>IGGLB low range</td>
<td>10 - 80 mg/L</td>
<td>25 μL</td>
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<tr>
<td>IGGHB high range</td>
<td>80 – 1600 mg/L</td>
<td>2 μL</td>
</tr>
<tr>
<td>IGGHD high + dilution</td>
<td>400 – 8000 mg/L, up to 160 g/L with higher dilution</td>
<td>40 μL</td>
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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 V should 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).

**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.

**Ordering information**

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack size</th>
<th>Catalog no.</th>
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<tbody>
<tr>
<td>IgG Bio</td>
<td>4 x 50 tests</td>
<td>06 681 743 001</td>
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<tr>
<td>IgG Bio HT</td>
<td>100 tests</td>
<td>06 608 540 001</td>
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<tr>
<td>Calibrator A Bio</td>
<td>6 x 1 ml</td>
<td>06 682 189 001</td>
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<tr>
<td>Control A Level 2 Bio</td>
<td>6 x 1 ml</td>
<td>06 682 227 001</td>
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<tr>
<td>Control A Level 3 Bio</td>
<td>6 x 1 ml</td>
<td>06 682 545 001</td>
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