INNOTEST® PHOSPHO-TAU(181P) is an ELISA microplate assay for the quantitative determination of human tau, phosphorylated at threonine 181, in human cerebrospinal fluid (CSF).

CSF-phospho-tau is a unique biomarker that can aid differentiation between Alzheimer’s disease and other primary dementias such as dementia with Lewy bodies (1,2).

**Article number:** 80317 (CE), 80062 (RUO)

**Number of tests:** 1 plate of 96 tests

The CE-marked kit allows easy, reliable, and specific quantification of CSF-phospho-tau\textsubscript{181P}. Higher specificity for Alzheimer’s disease versus non-AD dementia (eg, dementia with Lewy bodies) allows improved differential diagnosis.

**Features & Benefits:**

- User-friendly enzyme immunoassay
- Minimum number of handling steps
- Small sample volume: 75 µL CSF. Duplicate testing is strongly recommended and requires 2 x 75 µL CSF
- Reproducible results within the standards range
- Lowest detection limit: 15.6 pg/mL
  - Standards range: 15.6 to 500 pg/mL
  - Normal working range: 25 to 150 pg/mL

Click Poster INNOTEST® PHOSPHO-TAU(181P) to view the commercial poster.
Alzheimer's disease (AD) is one of the most important of all neuronal degenerative diseases because of its frequent occurrence (accounting for 60% of all senile dementias) (3) and devastating consequences. Given the gradual aging of the population and an exponential increase in the prevalence of AD after the age of 65 (from 5% at age 75-79 to 19% at age 85-89) (3, 4), the detection of AD is one of today's major challenges for healthcare. AD diagnosis is based on the presence of characteristic clinical features, the exclusion of other causes of dementia, and post-mortem confirmation. Current diagnostic criteria for probable AD, such as the NINCDS-ADRDA (5) or DSM-IV (6), lack the necessary specificity and sensitivity to discriminate AD from other causes of dementia such as vascular dementia (VAD), dementia with Lewy bodies (DLB), or co-existing Parkinson’s disease (PD). Clinico-pathological studies reveal diagnostic accuracy rates between 63% and 90% at academic medical centers (7). As specific therapeutic options are currently emerging, there is a definite need to seek means of improving diagnostic approaches.

The two most widely recognized pathological features of AD, although not 100% specific, are senile plaques and neurofibrillary tangles in the brain (8). These features also seem to correlate with the degree of dementia. Since biochemical changes in the brain are known to alter the composition of cerebrospinal fluid (CSF), tests based on the quantitative detection of these features in CSF may be helpful in the diagnosis of Alzheimer's disease.

Senile plaques, found in spaces between the brain's nerve cells, consist largely of an insoluble peptide called β-amyloid (Aβ). The main type of β-amyloid is Aβ1-42, a sticky protein fragment abnormally cleaved from a larger precursor protein called amyloid precursor protein (APP). Amyloid deposits are sparsely found in different regions of the normal aging brain, but become increasingly more abundant in the initial and subsequent stages of AD. As a result, β-amyloid1-42 concentrations in the CSF of AD patients are significantly lower compared to age-matched controls (9).

Neurofibrillary tangles are mainly composed of abnormal aggregations of tau protein. Normally, this protein is abundantly present in neurons where it serves to stabilize the microtubular network in the axons (10). In Alzheimer's disease, and particularly in those brain regions affected, the tau protein becomes abnormally phosphorylated, and this hyperphosphorylated form twists itself into paired helical filaments. These filaments aggregate to form the neurofibrillary tangles. When this happens, tau can no longer fulfil its stabilizing function, resulting in a collapse of the cell's internal transport, communication malfunction between nerve cells, and neuronal loss. Since tau is normally an intracellular protein, the amount found in cerebrospinal fluid (CSF) is low. However, in Alzheimer's disease, the slow neurodegenerative process leads to increased neuronal loss which may give rise to increased tau levels in CSF. The development of high affinity monoclonal antibodies highly specific for tau, tau phosphorylated at threonine 181, and specifically recognizing well-defined epitopes of Aβ has led to simple tests for the quantification of tau (INNOTEST® hTAU Ag) (11), phosphorylated tau (INNOTEST® PHOSPHO-TAU(181P)) (12), and Aβ1-42(INNOTEST® β- AMYLOID(1-42)) (13) in CSF.

Several recent studies have found significantly increased levels of CSF-phospho-tau in patients with AD (14-16), indicating that tau phosphorylation may well correlate with the loss of neuronal integrity. Importantly, other investigations have found normal levels of CSF-phospho-tau in both FTD and VAD.
as well as in common neurological (PD), cerebrovascular (acute stroke) and psychiatric (depression) disorders (17). More evidence that CSF-phospho-tau may prove to be valuable for differential diagnosis came from studies in which CSF-phospho-tau levels were significantly increased in AD patients as compared to DLB patients (1,2).

The value of CSF-tau, CSF- Aβ1-42, and CSF-phospho-tau in differential and early diagnosis has been described in several studies (18-21).

These tests may also prove to be of value in monitoring long-term effects of disease-modifying therapies.

References


Additional useful information about Alzheimer’s disease can be found on the following websites:

The Alzheimer forum site:
http://www.alzforum.org/new/detail.asp?id=875
http://www.alzforum.org/new/detail.asp?id=804
http://www.alzforum.org/new/detail.asp?id=709

Related products
INNOTEST® hTAU Ag
INNOTEST® β -AMYLOID(1-42)

Publications

- Discrimination of dementia with Lewy bodies from Alzheimer’s disease via determination of the phospho-tau concentration in cerebrospinal fluid
- CSF markers for the diagnosis of Alzheimer’s disease.
- CSF-phospho-tau (181P) as a promising marker for discriminating Alzheimer’s disease from dementia with Lewy bodies.
- Effects of cholinesterase inhibitors on Alzheimer’s disease biomarkers.
- Phosphorylated tau in cerebrospinal fluid as marker for Creutzfeldt-Jacob disease.
- Total tau and phosphorylated tau 181 levels in the cerebrospinal fluid of patients with frontotemporal dementia due to P301L and G272V tau mutations.
- Value of CSF beta-amyloid(1-42) and tau as predictors of Alzheimer’s disease in patients with mild cognitive impairment.
- CSF markers for incipient Alzheimer’s disease.
- Standardization and evaluation of INNOTEST® PHOSPHO-TAU(181P) for the detection of tau, phosphorylated at threonine 181, in cerebrospinal fluid.

Customer support

For all inquiries regarding Innogenetics products and instrumentation, we offer the services of our customer support team. This team of technical experts is committed to dealing with

- General inquiries
- Technical assistance
- Complaint resolution

The customer support team is designed to be your principle point of contact. They will ensure that the appropriate person handles all product-related inquiries. For this service please contact us via e-mail, call us (+32-9-329-1611), or fax (+32-9-329-1775).
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Symbols used

Manufactured by

For research use only
Not for use in diagnostic procedures

Lot number

Catalog number

Use by

Consult instructions for use

*INNOGENETICS® is a Registered Trademark of Innogenetics N.V.
**English**

**Intended use**

The INNOTEST® PHOSPHO-TAU\(_{(181P)}\) is a solid-phase enzyme immunoassay for the quantitative determination of phosphorylated tau (PHOSPHO-TAU\(_{(181P)}\)) in human cerebrospinal fluid (CSF).

For research use only. Not for use in diagnostic procedures.
Test principle

The INNOTEST® PHOSPHO-TAU$_{(181P)}$ is a solid-phase enzyme immunoassay in which the phosphorylated tau protein or fragments are captured by a first monoclonal antibody, HT7. CSF samples are added and incubated with a biotinylated antibody, AT270bio. This antigen-antibody complex is then detected by a peroxidase-labeled streptavidin. After addition of substrate solution, positive samples will develop en blue color. The reaction is stopped by the addition of sulfuric acid that produces a yellow color. The absorbance is then measured at 450 nm.

Reagents

*Description, preparation for use and recommended storage conditions*

- If kept at 2 - 8°C, and stored in the original vials, the reagents, opened or unopened, are stable until the expiry date of the kit. Do not use the reagents beyond the expiry date.
- All reagents and the aluminum foil bag containing the strips, must be brought to room temperature (18 - 30°C) approximately 30 minutes before use and should be returned to the refrigerator immediately after use. To avoid water condensation into the wells, the aluminum foil bag must be kept closed until the strips are stabilized at room temperature.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Ref.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coated Plate</td>
<td>1 x 96</td>
<td>55515</td>
<td>1 sealed bag containing a strip holder with 12 x 8 coated test wells and a silicagel bag as desiccant.</td>
</tr>
</tbody>
</table>
| Sample Diluent  | 1 x 30 ml| 55992 | Phosphate buffer with stabilizing proteins and 0.01% MIT/0.1% CAA as preservative, used to dilute the standard and samples with high concentrations of PHOSPHO-TAU$_{(181P)}$.
<table>
<thead>
<tr>
<th>Item</th>
<th>Concentration</th>
<th>Volume</th>
<th>Catalog Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugate 1 100x</td>
<td>1 x 0.3 ml</td>
<td>57205</td>
<td>Mouse anti-PHOSPHO-TAU ((181P)) IgG labeled with biotin (AT270Bio) in phosphate buffer with stabilizing proteins and 0.05% Proclin 300 as preservative. Dilute 100x with Conjugate Diluent 1 before use (see preparation of reagents). Conjugate working solution 1 must be prepared freshly for each test.</td>
<td></td>
</tr>
<tr>
<td>Conjugate 2 100x</td>
<td>1 x 0.3 ml</td>
<td>55990</td>
<td>Peroxidase-labeled streptavidin containing 0.02% MIT and 0.02% bromonitrodioxane as preservative. Dilute 100x with Conjugate Diluent 2 before use (see preparation of reagents). Conjugate working solution 2 must be prepared freshly for each test.</td>
<td></td>
</tr>
<tr>
<td>Conjugate Diluent 1</td>
<td>1 x 20 ml</td>
<td>55989</td>
<td>Phosphate buffer with stabilizing proteins and 0.01% MIT/0.1% CAA as preservative (purple colored buffer solution), used to dilute Conjugate 1.</td>
<td></td>
</tr>
<tr>
<td>Conjugate Diluent 2</td>
<td>1 x 20 ml</td>
<td>55991</td>
<td>Phosphate buffer containing 0.05% Proclin 300 as preservative (green colored buffer solution), used to dilute the Conjugate 2.</td>
<td></td>
</tr>
<tr>
<td>Substrate TMB 100x</td>
<td>1 x 0.3 ml</td>
<td>55993</td>
<td>Tetramethyl benzidine (TMB) dissolved in dimethyl sulfoxide (DMSO). Dilute 100x in Substrate Buffer before use. (see preparation of reagents). Substrate working solution must be prepared freshly for each test.</td>
<td></td>
</tr>
<tr>
<td>Substrate Buffer</td>
<td>1 x 30 ml</td>
<td>51175</td>
<td>Phosphate-citrate buffer containing 0.02% hydrogen peroxide, used to dilute the Substrate TMB.</td>
<td></td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 30 ml</td>
<td>55995</td>
<td>0.9N sulfuric acid.</td>
<td></td>
</tr>
<tr>
<td>Wash Solution 25x</td>
<td>1 x 60 ml</td>
<td>51613</td>
<td>Phosphate buffer containing 0.01% MIT/0.09% CAA, to be diluted 25x with distilled or deionized water before use. Prepare at least 40 ml of diluted wash solution for each test well strip. Salt crystals may be formed in the concentrated wash solution after storage at 2-8°C. These crystals must be completely redissolved. Diluted wash solution is stable for 4 weeks if kept at 2-8°C.</td>
<td></td>
</tr>
</tbody>
</table>
Preparation of reagents

Preparation of conjugate working solution 1 and 2 and substrate working solutions

<table>
<thead>
<tr>
<th>Number of tests</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONJ 1 in µl</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>CONJ DIL 1 in ml</td>
<td>1</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of tests</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONJ 2 in µl</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>80</td>
<td>120</td>
</tr>
<tr>
<td>CONJ DIL 2 in ml</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of tests</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUBS in µl</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>80</td>
<td>120</td>
</tr>
<tr>
<td>SUBS BUF in ml</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>

Preparation of diluted wash solution

<table>
<thead>
<tr>
<th>Number of tests</th>
<th>8 wells</th>
<th>16 wells</th>
<th>32 wells</th>
<th>64 wells</th>
<th>96 wells</th>
</tr>
</thead>
<tbody>
<tr>
<td>WASH SOLN</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>H₂O 1 in ml</td>
<td>120</td>
<td>240</td>
<td>480</td>
<td>960</td>
<td>1440</td>
</tr>
</tbody>
</table>

Materials required but not provided

- Distilled or deionized water.
- Calibrated precision pipettes with disposable tip to deliver volumes in the ranges of 10 - 1000 µl. A calibrated multi-channel pipette to deliver 25 µl, 50 µl, 75 µl, 100 µl, 200 µl is recommended for addition of samples, conjugate working solutions, Substrate working solution and Stop Solution.
- Vortex mixer or equivalent.
- Polypropylene tubes to dilute the samples and to make the standards.
- Microplate washer; alternatively, washing can be performed by using a repeat pipette delivering 0.4 ml volumes and an aspirating device.
- Timer.
- Absorbent tissues.
- Microplate reader with 450 ± 5 nm filter, optionally, with 595 nm, 620 nm or 690 nm filter for dual wavelength analysis, and with a linear absorbency range of 0 to 3.000 or higher.
- Disposable vials for preparation of working solutions.
- Appropriate biohazard waste containers for potentially contaminated materials.
- Microplate shaker (1000 rpm); alternatively, mixing can be performed by tapping the side of the plate.

Safety and environment

- Please refer to the Material Safety Data Sheet (MSDS) and product labelling for information on potentially hazardous components. The most recent MSDS version is available on the website www.innogenetics.com.

R43, S24-37
Irritant! (Xi) Avoid contact with skin. May cause sensitization by skin contact. Wear suitable gloves. **Contains 0.1% 2-Chloroacetamide:** CONJ DIL 1, SAMP DIL, PHOSPHO-TAU\(^{(181P)}\), STAND.

R36/37/38, S23-24-26
Irritant! (Xi) Irritating to eyes, respiratory system and skin. Do not breathe vapour. Avoid contact with skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. **Contains DMSO:** SUBS TMB 100x.
Corrosive! (C) Causes burns. After contact with skin, wash immediately with plenty of soap and water. Wear suitable protective clothing, gloves, and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). **Contains H\textsubscript{2}SO\textsubscript{4}:** STOP SOLN.

- Specimens should always be handled as potentially infectious. All biological materials should be considered as being potentially infectious and should be handled as such. Only adequately trained personnel should be permitted to perform the test procedure. All biological materials should be disposed of in accordance with established safety procedures.
  - Autoclave for at least 15 minutes at 121°C.
  - Incinerate disposable material.
  - Mix liquid waste with sodium hypochlorite so that the final concentration is ± 1% sodium hypochlorite. Allow to stand overnight before disposal. **CAUTION:** Neutralize liquid waste that contains acid before adding sodium hypochlorite.

- CONJ 2 contains MIT/Bromonitrodioxane as preservative and CONJ DIL 2, CONJ1 100x contain Proclin 300 as preservative.
- The kit contains 0.9 N sulfuric acid as Stop Solution.
- Use of personal protective equipment is necessary: gloves and safety spectacles when manipulating dangerous or infectious agents.
- Waste should be handled according to the institution’s waste disposal guidelines. All federal, state, and local environmental regulations should also be observed.

**REMARK:**
Special precautions for Transmissible Spongiform Encephalopathy (TSE)/Prion contaminated materials:

- **Inactivation of samples**
  Clinical samples, e.g. CSF, should be autoclaved or immersed in a solution of sodium hypochlorite resulting in 20,000ppm free chlorine for 1 hour before final disposal by incineration.
**Waste disposal**

All material classified as clinical waste should be disposed of by incineration at an authorized incineration site. For the safe handling of clinical waste, use secure leak-proof containers, e.g. double bagging, where appropriate. Avoid external contamination of the container.

**REFERENCE:**
- Advisory Committee on Dangerous Pathogens (UK) - Spongiform Encephalopathy Advisory Committee - Transmissible Spongiform Encephalopathy Agents: Safe Working and the Prevention of Infection

**Specimens**

- Human cerebrospinal fluid stored at -20°C (preferably -80°C), collected in polypropylene tubes. The specimens must be free of cells and show no hemolysis.
- The assay is not made for serum screening or cell culture supernatants.
- It is recommended to aliquot the samples to avoid repeated freeze/thaw cycles.
- CSF samples with an OD$_{450\text{nm}}$ value above the highest standard should be diluted in Sample Diluent to determine the correct levels.
- Insoluble material should be removed from all samples by centrifugation before testing, e.g. centrifuge at 4000 g for 10 minutes.

**Remarks and precautions**

- Do not use the kit beyond the expiry date.
- Do not mix components from kits with different lot numbers.
- All vessels used to prepare conjugate and substrate working solutions must be cleaned thoroughly to avoid contamination.
- Do not touch the top of the ELISA plates with your fingers to avoid contamination.
- Avoid microbial contamination of reagents.
- Ensure that samples and diluted standard solutions are homogeneous before use.
- Use a new pipette tip for each specimen.
- Ensure that specimen is added to the microwell. Addition of specimens (but not the standard and blank) should be verified visually using the color change (purple color is shifting to blue).
- To avoid contamination, do not touch the edge of the wells with the pipette tips when adding sample or conjugate working solution.
- Remove any air bubbles present by tapping the microtiter plate gently or by mixing on plate shaker for 1 minute at 1000 rpm.
- Do not expose substrate working solution to strong light during incubation or storage. **Place the plate in the dark during the incubation of the substrate.** The prepared substrate working solution must be colorless when used; if the solution turns blue it must be replaced.
- Stop Solution, substrate working solution, conjugate working solution 1 or conjugate working solution 2 should not be in contact with metals or metal ions to avoid unwanted color formation.
- If the wells cannot be filled with conjugate or substrate immediately after washing, place them upside down on an absorbent tissue, soaked in Wash Solution, for no longer than 15 minutes.
- Do not use blood collection tubes for the preparation of the reagent working solutions.

**Washing procedure**

In house washing was performed with an automatic washer: Columbus M8/2ch from Tecan. The protocol is available upon request.

For other washers or protocols, carry out **automatic washing** as follows:
- Pre-rinse the washer with wash solution.
- Perform 5 wash cycles ensuring that:
  - the fill volume is 400µl/well
  - the dispensing height is set to completely fill the well
  - the time taken to complete one aspiration/wash/soak cycle is approximately 30 seconds.

Perform **manual wash** as follows:
- Aspirate completely the liquid from all wells by lowering an aspiration tip (aspiration device) gently to the bottom of each well.
- Take care not to scratch the inside of the well surface.
- After aspiration, fill the wells with 400 µl of diluted wash solution.
- Remove the liquid from the wells.
- Perform the steps five times.
- After the washing procedure, invert the plate and tap dry on absorbent tissue.

Incomplete washing will adversely affect the test outcome. Contamination of wash solution and washer can cause extensive problems. In case problems occur, disinfect the wash bottles and washer overnight with an appropriate disinfectant solution and rinse with water.

**Test procedure**

Please read 'Remarks and precautions' before performing the test.

**NOTE:**
- Allow all specimens and test reagents to reach room temperature (18°C - 30°C) before use.
- Have all reagents and specimens ready before starting the assay. Once the test has started, it must be performed without any interruption in order to achieve the most reliable and consistent results.

1. Place the required number of **strips** into the strip-holder for each test run, duplicate wells of the 6 standards and the blank should be prepared. The strips can be marked on one edge for identification. Place any unused strips in the plastic minigrip bag with the silicagel desiccant.

2. Prepare **conjugate working solution 1** and add **25 µl** to each well of the antibody-coated plate.

3. Add **75 µl of each standard (including the blank of 75 µl Sample Diluent) and the samples** to duplicate wells. CSF samples should be vortexed before testing. **Mix** gently by tapping the side of the plate or by shaking 1 minute at 1000 rpm. **Cover** the strips with an adhesive sealer and **incubate** overnight (14 - 18 hours) in an incubator at 2 - 8°C.

4. Prepare conjugate working solution 2 just before the end of step 3.

5. **Wash** each well **5 times** (see Directions for washing).

6. Add **100 µl conjugate working solution 2** to each well. **Cover** the strips with a new adhesive sealer and **incubate** for 60 ± 5 minutes at room temperature (18 - 30°C).
7. Prepare substrate working solution just before the end of step 6.
8. **Wash** each well **5 times** (see Directions for washing).
9. Add **100 µl** of **substrate working solution** to each well and **incubate** for **30 ± 3 minutes** at room temperature (18 - 30°C) in the dark.
10. Add **50 µl** of **Stop Solution** to each well, in the same sequence and at the same time intervals as the substrate working solution. Tap the stripholder carefully to ensure thorough mixing.
11. **Read** (within 15 minutes after step 7) the absorbance at 450 nm (single wavelength). For dual wavelength analysis, 690 nm or 620 nm can be used as the reference wavelength.

**Results**

**Validation**

- When CSF samples are added the conjugate working solution should change from purple to blue.
- The absorbency at 450 nm (single wavelength) of the individual blanks (Sample Diluent) should be lower than 0.100.

**NOTE:**

- Absorbency values for dual wavelength (450 nm, 595 nm, 620 nm and 690 nm) analysis differ about 50 mOD from single wavelength values, without affecting the final outcome of the test.
- Based on internal and external data, the OD-value for the highest standard (500 pg/ml) was at least 2.3 OD. However, this value can depend on the OD-reader used, and therefore cannot be used as a real validation criterion.

**Test results**

Calculate the mean absorbency for the standard solutions and the unknown samples. Repeat the test if individual OD values differ by more than 20%.

Construct the standard curve by plotting the mean absorbency values obtained for each of the standard solutions on the vertical (Y) axis versus the corresponding concentrations on the horizontal (X) axis. Draw the best fitting curve through these points.
NOTE:
- A sigmoidal curve fitting is recommended (sigmoidal dose-response with variable slope, four parameter logistic equation or Logit-Log).

Using the mean absorbency value of each unknown CSF sample, determine the corresponding concentration of PHOSPHO-TAU\(_{(181P)}\) in pg/ml.

The concentrations of samples can only be determined if the absorbency is within the limits of the standard curve. Fitting of values, which lie above the highest point or below the lowest point of the standard curve, can lead to aberrantly calculated concentrations.

**Limitations of the procedure**

- The INNOTEST® PHOSPHO-TAU\(_{(181P)}\) assay procedure was designed to quantify PHOSPHO-TAU\(_{(181P)}\) in human cerebrospinal fluid. Insufficient data are available to interpret tests performed on other body fluids or brain tissue samples. Therefore, testing of such specimens is not recommended.

**Test performance**

**Precision**

Precision was determined based on internal evaluation of 3 consecutive batches and external evaluation of one batch. Precision is expressed by the imprecision value %CV on concentrations, which was found to be lower than 10% in the region from ± 25 pg/ml (LLOQ, lower limit of quantitation) to 250 pg/ml (ULOQ, upper limit of quantitation) and was typically lower than 5% in the normal working region [25 - 150 pg/ml]. The LOD (limit of detection) was close to the lowest standard of 15.6 pg/ml.

Repeatability %CV was repeatedly found to be less than 5%. Interlot (R) variability was lower than 10%.

Inter-laboratory trial variability (5 labs; 3 samples of different concentration levels in the normal working region) also showed %CV < 5% for within-lab variability and %CV for between-lab variability of 10% on average, at the three concentration levels.
Troubleshooting

**OD values too low**

**Possible cause**
Incorrect storage of CSF samples.

**Solution**
- Store CSF at -20°C or lower for longer time periods.
- Use polypropylene tubes for collection and storage of CSF.

**Possible cause**
Components from different lots were mixed.

**Solution**
- Repeat the test using components from the same lot.

**Possible cause**
Pipetting automate or pipets not OK.

**Solution**
- Use only calibrated pipets.

**OD values too high for samples and blanks**

**Possible cause**
Contamination of Conjugate working solution 2 and Substrate.

**Solution**
- Use clean containers for the preparation of Conjugate working solution 2 and Substrate.

**Poor reproducibility**

**Possible cause**
Contaminated pipette tips.

**Solution**
- Use a clean tip for each sample.

**Possible cause**
Contamination of microtiter plate by opening of sample container over the plate.

**Solution**
- Avoid opening of sample containers over the microtiter plate.

**Possible cause**
Contamination from one well to another during removal of the adhesive plate sealer.

**Solution**
- Remove carefully the plate sealer and use a new one for each incubation step.

**Possible cause**
Contamination of washer.

**Solution**
- Disinfect Wash Solution container and washer with an appropriate disinfectant and rinse with water.

**Possible cause**
Samples and controls were not adequately mixed with the sample diluent.

**Solution**
- Samples and controls have to be completely mixed with the Sample Diluent on the microtiter plate shaker at 1000 rpm for one minute or by tapping gently the side of the plate.