HUMAN GFAP ELISA

Product Data Sheet

Cat. No.: RD192072200R

For Research Use Only
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This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.

Use only the current version of Product Data Sheet enclosed with the kit!
1. INTENDED USE

The RD192072200R Human GFAP ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Glial Fibrillary Acidic Protein (GFAP).

Features

- **It is intended for research use only**
- The total assay time is about 5 hours
- The kit measures GFAP in serum, cerebrospinal fluid (CSF) and plasma
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is purified native protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.
3. INTRODUCTION

Glial Fibrillary Acidic Protein (GFAP), as a member of the cytoskeletal protein family, is the principal 8-9 nm intermediate filament in mature astrocytes of the central nervous system (CNS). GFAP is a monomeric molecule with a molecular mass between 40 and 53 kDa and an isoelectric point between 5.7 and 5.8. GFAP is highly brain specific protein that is not found outside the CNS. Some studies showed that GFAP is released into the blood very soon after traumatic brain injury (TBI), that GFAP is related to brain injury severity and outcome after TBI and that GFAP is not released after multiple trauma without brain injury.

In the CNS following injury, either as a result of trauma, disease, genetic disorders, or chemical insult, astrocytes become reactive and respond in a typical manner, termed astrogliosis. Astrogliosis is characterized by rapid synthesis of GFAP. GFAP normally increases with age and there is a wide variation in collection and processing of human brain tissue. Thanks to the high brain specificity and early releasing from CNS after TBI, GFAP might be suitable marker for early diagnostics.

Areas of investigation:
- Brain injury
- Ischemic stroke

4. TEST PRINCIPLE

In the BioVendor Human GFAP ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human GFAP antibody. After 120 minutes incubation and washing, biotin labelled monoclonal anti-human GFAP antibody is added and incubated for 60 minutes with captured GFAP. After another washing, streptavidin-HRP conjugate is added. After 60 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of GFAP. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.
5. PRECAUTIONS

- For professional use only
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
  - The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements
7. REAGENT SUPPLIED

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>State</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Coated Microtiter Strips</td>
<td>ready to use</td>
<td>96 wells</td>
</tr>
<tr>
<td>Biotin Labelled Antibody</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Streptavidin-HRP Conjugate</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Master Standard</td>
<td>lyophilized</td>
<td>1 vial</td>
</tr>
<tr>
<td>Quality Control HIGH</td>
<td>lyophilized</td>
<td>1 vial</td>
</tr>
<tr>
<td>Quality Control LOW</td>
<td>lyophilized</td>
<td>1 vial</td>
</tr>
<tr>
<td>Standard Diluent</td>
<td>ready to use</td>
<td>9 ml</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Wash Solution Conc. (10x)</td>
<td>concentrated</td>
<td>100 ml</td>
</tr>
<tr>
<td>Substrate Solution</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Product Data Sheet + Certificate of Analysis</td>
<td>-</td>
<td>1 pc</td>
</tr>
</tbody>
</table>

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Vortex mixer
- Glassware (graduated cylinder and bottle) for Wash Solution
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)
9. PREPARATION OF REAGENTS

- All reagents need to be brought to room temperature prior to use
- Always prepare only the appropriate quantity of reagents for your test
- Do not use components after the expiration date marked on their label
- Assay reagents supplied ready to use:

  Antibody Coated Microtiter Strips
  Stability and storage:
  Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 month stored at 2-8°C and protected from the moisture.

  Standard Diluent
  Dilution Buffer
  Biotin Labelled Antibody
  Streptavidin-HRP Conjugate
  Substrate Solution
  Stop Solution
  Stability and storage:
  Opened reagents are stable 3 months when stored at 2-8°C.

- Assay reagents supplied concentrated or lyophilized:

  Human GFAP Master Standard
  Refer to the Certificate of Analysis for current volume of Standard Diluent needed for reconstitution of standard!!!
  Reconstitute the lyophilized Master Standard with Standard Diluent just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the GFAP in the stock solution is \(25\) ng/ml. Prepare set of standards using Standard Diluent as follows:
<table>
<thead>
<tr>
<th>Volume of Standard</th>
<th>Standard Diluent</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>-</td>
<td>25 ng/ml</td>
</tr>
<tr>
<td>300 µl of stock</td>
<td>450 µl</td>
<td>10 ng/ml</td>
</tr>
<tr>
<td>300 µl of 10 ng/ml</td>
<td>300 µl</td>
<td>5 ng/ml</td>
</tr>
<tr>
<td>300 µl of 5 ng/ml</td>
<td>300 µl</td>
<td>2.5 ng/ml</td>
</tr>
<tr>
<td>300 µl of 2.5 ng/ml</td>
<td>450 µl</td>
<td>1 ng/ml</td>
</tr>
<tr>
<td>300 µl of 1 ng/ml</td>
<td>300 µl</td>
<td>0.5 ng/ml</td>
</tr>
<tr>
<td>300 µl of 0.5 ng/ml</td>
<td>300 µl</td>
<td>0.25 ng/ml</td>
</tr>
</tbody>
</table>

Dilute prepared Standards (25 - 0.25 ng/ml) 3x with Standard Diluent just prior to the assay, e.g. 100 µl of Standard + 200 µl of Standard Diluent for duplicates.

**Stability and storage:**
Set of standards (25 - 0.25 ng/ml) should be aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

**Do not store the diluted Standard solutions.**

**Quality Controls HIGH, LOW**
Refer to the Certificate of Analysis for current volume of distilled water needed for reconstitution and for current Quality Controls concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with distilled water just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Dilute reconstituted Quality Controls 3x with Dilution Buffer, e.g. 50 µl of Quality Control + 100 µl of Dilution Buffer when assaying samples in singlets, or preferably 100 µl of Quality Control + 200 µl of Dilution Buffer for duplicates.

**Stability and storage:**
The reconstituted Quality Controls must be used immediately or stored frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

**Do not store the diluted Quality Controls.**

**Wash Solution Conc. (10x)**
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution, e.g. 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

**Stability and storage:**
The diluted Wash Solution is stable for 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.
10. PREPARATION OF SAMPLES

The kit measures GFAP in serum, plasma and CSF.

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 3x with Dilution Buffer just prior to the assay, e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets, or preferably 100 µl of sample + 200 µl of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/thaw cycles.

Do not store the diluted samples.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.
11. ASSAY PROCEDURE

1. Pipet 100 µl of diluted Standards, Quality Controls, Standard Diluent (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for 2 hours, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 µl of Biotin Labelled Antibody Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100 µl of Conjugate Solution into each well.
8. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add 100 µl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for 10-15 minutes at room temperature. The incubation time may be extended [up to 30 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding 100 µl of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 12.

Note 1: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine GFAP concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.
<table>
<thead>
<tr>
<th></th>
<th>strip 1+2</th>
<th>strip 3+4</th>
<th>strip 5+6</th>
<th>strip 7+8</th>
<th>strip 9+10</th>
<th>strip 11+12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Standard 25</td>
<td>QC HIGH</td>
<td>Sample 7</td>
<td>Sample 15</td>
<td>Sample 23</td>
<td>Sample 31</td>
</tr>
<tr>
<td>B</td>
<td>Standard 10</td>
<td>QC LOW</td>
<td>Sample 8</td>
<td>Sample 16</td>
<td>Sample 24</td>
<td>Sample 32</td>
</tr>
<tr>
<td>C</td>
<td>Standard 5</td>
<td>Sample 1</td>
<td>Sample 9</td>
<td>Sample 17</td>
<td>Sample 25</td>
<td>Sample 33</td>
</tr>
<tr>
<td>D</td>
<td>Standard 2.5</td>
<td>Sample 2</td>
<td>Sample 10</td>
<td>Sample 18</td>
<td>Sample 26</td>
<td>Sample 34</td>
</tr>
<tr>
<td>E</td>
<td>Standard 1.0</td>
<td>Sample 3</td>
<td>Sample 11</td>
<td>Sample 19</td>
<td>Sample 27</td>
<td>Sample 35</td>
</tr>
<tr>
<td>F</td>
<td>Standard 0.5</td>
<td>Sample 4</td>
<td>Sample 12</td>
<td>Sample 20</td>
<td>Sample 28</td>
<td>Sample 36</td>
</tr>
<tr>
<td>G</td>
<td>Standard 0.25</td>
<td>Sample 5</td>
<td>Sample 13</td>
<td>Sample 21</td>
<td>Sample 29</td>
<td>Sample 37</td>
</tr>
<tr>
<td>H</td>
<td>Blank</td>
<td>Sample 6</td>
<td>Sample 14</td>
<td>Sample 22</td>
<td>Sample 30</td>
<td>Sample 38</td>
</tr>
</tbody>
</table>

*Figure 1: Example of a work sheet.*
12. **CALCULATION**

Most microplate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of GFAP ng/ml in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

Samples, Quality Controls and Standards are all diluted 3x prior to analysis, so there is no need to take this dilution factor into account.

*Figure 2: Typical Standard curve for Human GFAP ELISA.*
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human GFAP ELISA are presented in this chapter

- **Sensitivity**
  Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is calculated from the real GFAP values in wells and is 0.045 ng/ml.
  *Dilution Buffer is pipetted into blank wells.

- **Limit of assay**
  Results exceeding GFAP level of 25 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the GFAP concentration.

- **Specificity**
  The antibodies used in this ELISA are specific for human GFAP with no detectable crossreactivities to human S100A1, S100B, S100P, S100Z and neuroglobin at 100 ng/ml.

- **Precision**
  Intra-assay (Within-Run) (n=8)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.27</td>
<td>0.082</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>0.43</td>
<td>0.027</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Inter-assay (Run-to-Run) (n=7)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (ng/ml)</th>
<th>SD (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.62</td>
<td>0.160</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>0.40</td>
<td>0.023</td>
<td>5.2</td>
</tr>
</tbody>
</table>
• **Spiking Recovery**
Serum samples were spiked with different amounts of human GFAP and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.04</td>
<td>7.04</td>
<td>111.2</td>
</tr>
<tr>
<td></td>
<td>7.83</td>
<td>5.04</td>
<td>102.0</td>
</tr>
<tr>
<td></td>
<td>5.10</td>
<td>2.54</td>
<td>100.9</td>
</tr>
<tr>
<td></td>
<td>2.56</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.41</td>
<td>5.41</td>
<td>113.6</td>
</tr>
<tr>
<td></td>
<td>6.11</td>
<td>1.41</td>
<td>114.7</td>
</tr>
<tr>
<td></td>
<td>1.62</td>
<td>0.91</td>
<td>110.7</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>5.00</td>
<td>104.0</td>
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<tr>
<td></td>
<td>0.85</td>
<td>1.00</td>
<td>85.2</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>0.50</td>
<td>83.4</td>
</tr>
</tbody>
</table>

• **Linearity**
Serum samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>23.54</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>13.00</td>
<td>11.77</td>
<td>110.4</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>6.66</td>
<td>5.89</td>
<td>113.1</td>
</tr>
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<td></td>
<td>8x</td>
<td>3.34</td>
<td>2.94</td>
<td>113.5</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>8.59</td>
<td>-</td>
<td>-</td>
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<td>2x</td>
<td>4.16</td>
<td>4.30</td>
<td>96.8</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>2.25</td>
<td>2.15</td>
<td>104.9</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>1.10</td>
<td>1.07</td>
<td>102.3</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>5.34</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>2.33</td>
<td>2.67</td>
<td>87.1</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>1.22</td>
<td>1.34</td>
<td>91.6</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>0.57</td>
<td>0.67</td>
<td>85.9</td>
</tr>
</tbody>
</table>

• **Reference Ranges**
It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for GFAP levels with the assay.
14. DEFINITION OF THE STANDARD

The native protein purified from normal human brain is used as the Standard in this assay. GFAP is a monomeric molecule with a molecular mass between 40 and 53 kDa and an isoelectric point between 5.7 and 5.8.

15. METHOD COMPARISON

The BioVendor Human GFAP ELISA was not compared to other commercial immunoassays.

16. TROUBLESHOOTING AND FAQS

Weak signal in all wells
Possible explanations:
- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells
Possible explanations:
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)
Possible explanation:
- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples
17. REFERENCES

References to GFAP:

References to this product:

- Steiner J et al.: Increased cerebrospinal fluid and serum levels of S100B in first-onset schizophrenia are not related to a degenerative release of glial fibrillar acidic protein, myelin basic protein and neurone-specific enolase from glia or neurones. *J Neurol Neurosurg Psychiatry.* 77(11): 1284-7 (2006)

For more references on this product see our WebPages at [www.biovendor.com](http://www.biovendor.com)
## 18. EXPLANATION OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>REF</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>Cont.</td>
<td>Content</td>
</tr>
<tr>
<td>LOT</td>
<td>Lot number</td>
</tr>
<tr>
<td><img src="image" alt="Warning symbol" /></td>
<td>Attention, see instructions for use</td>
</tr>
<tr>
<td><img src="image" alt="Biohazard symbol" /></td>
<td>Potential biological hazard</td>
</tr>
<tr>
<td><img src="image" alt="Exclamation point" /></td>
<td>Expiry date</td>
</tr>
<tr>
<td><img src="image" alt="Temperature symbol" /></td>
<td>Storage conditions</td>
</tr>
<tr>
<td><img src="image" alt="Manufacturing symbol" /></td>
<td>Name and registered office of the manufacturer</td>
</tr>
</tbody>
</table>
Antibody Coated Microtiter Strips

Reconstitute QCs and Master Standard and prepare set of Standards

Dilute QCs and Standards 3x

Add Standards, QCs and samples 100 µl

Dilute samples 3x

Incubate at RT for 2 hour/300 rpm

Prepare Wash Solution

Wash 3x

Add Biotin Labelled Antibody 100 µl

Incubate at RT for 1 hour / 300rpm

Add Streptavidin-HRP Conjugate 100 µl

Incubate at RT for 1 hour / 300rpm

Add Substrate Solution 100 µl

Incubate at RT for 10-15 min

Add Stop Solution 100 µl

Read absorbance and calculate results

Assay Procedure Summary