This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.

Development of the product was supported by Ministry of Industry and Trade of the Czech Republic, project No. FR-TI3/666.

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

The Human Prostate Cancer Assay 1 is a fluorescent bead based immunoassay for the quantitative measurement of human PSP94. This singleplex protein assay is characterized by high sensitivity and wide dynamic range of the measurement.

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

Prostate secretory protein of 94 amino acids (PSP94), also known as β-microseminoprotein or prostatic inhibin-like protein, is a small, nonglycosylated peptide consisting of 94 amino acids with molecular mass 10.7 kDa, and is one of the major secretory proteins of the prostate glands. PSP94 is synthesized as a preprotein of 114 amino acid residues, from which a 20-residue signal peptide is cleaved off to form the mature protein.

PSP94 along with PSA (Prostate-specific Antigen) and PAP (Prostate Acid Phosphatase) are the three most abundant proteins in seminal fluid. As with other prostate-secreted proteins, PSP94 can leak into the blood upon benign or malignant prostate epithelial disruption and can be measured within serum. PSP94 is not solely synthesized by the prostate epithelium, as the protein can also be detected in nonreproductive organs such as in the respiratory and gastrointestinal tracts, of which, the gastric mucosa shows particularly high expression. Accordingly, PSP94 can be measured in serum of both men and women, but the serum levels in women were found to be around two-thirds of those measured in men. PSP94 forms high-affinity complexes with two related Cys-rich proteins: PSP94-binding protein in blood plasma and cysteine-rich secretory protein 3 (CRISP-3) in semen.

Evidence suggest that PSP94 has systemic functions including growth regulation and induction of apoptosis in prostate cancer cells in vitro and in vivo, and regulation of calcium levels during hypercalcemia secondary to malignancy. Several studies have demonstrated a progressive decrease in PSP94 expression as prostate cancer progresses from a hormone-dependent to a hormone-independent state with complete lack of PSP94 production in highly advanced metastatic prostate cancer. This differential expression could make PSP94 a prognostic clinical marker for prostate cancer and could help distinguish patients with aggressive forms of prostate cancer. A recent study demonstrated a close correlation between PSP94 in serum and seminal plasma, supporting the potential use of PSP94 as a serum marker of prostate secretory function as well.

Clinical use and areas of investigation:
Prostate cancer
4. TEST PRINCIPLE

In the BioVendor Human Prostate Cancer Assay 1, coupled beads are inserted into the wells and immediately washed. Then standards and samples can be pipetted and incubated in microplate wells. After 30 minutes incubation and washing, biotin-labelleld anti-human PSP94 antibody is added and incubated for another 30 minutes. After another washing, streptavidin - R phycoerytrin conjugate is added. After 10 minutes incubation and the last washing step, detection buffer is uploaded. Applied Bio code reader quantifies the fluorescent value of all beads for each bead type in the well. Beads for individual analytes contain specific barcode, which determines the bead identity. The intensity of phycoerytrin fluorescence intensity is proportional to the concentration of each analyte. A standard curve is constructed by plotting fluorescence values against concentrations of standards, 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 animal origin. These materials should be handled as potentially infectious
- Streptavidin - R Phycoerytrin Conjugate is light sensitive. Protect it from light to avoid photobleaching of the label
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware and polypropylene tubes
- 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
- 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>96-well Flat Bottom Microtiter Plate</td>
<td>ready to use</td>
<td>96 wells</td>
</tr>
<tr>
<td>Human Prostate Cancer Assay 1 Antibody Coated Beads (50x)</td>
<td>concentrated</td>
<td>120 ul / 1 vial</td>
</tr>
<tr>
<td>Biotin Labelled Antibody Conjugate (50x)</td>
<td>concentrated</td>
<td>60 ul / 1 vial</td>
</tr>
<tr>
<td>Streptavidin – R Phycoerytrin Conjugate (100x)</td>
<td>concentrated</td>
<td>60 ul / 1 vial</td>
</tr>
<tr>
<td>Detection Buffer</td>
<td>ready to use</td>
<td>20 ml</td>
</tr>
<tr>
<td>Human Prostate Cancer Assay 1 Master Standard</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>ready to use</td>
<td>10 ml</td>
</tr>
<tr>
<td>Biotin Diluent</td>
<td>ready to use</td>
<td>5 ml</td>
</tr>
<tr>
<td>Streptavidin – R Phycoerytrin Diluent</td>
<td>ready to use</td>
<td>10 ml</td>
</tr>
<tr>
<td>Wash Solution Conc. (10x)</td>
<td>concentrated</td>
<td>100 ml</td>
</tr>
<tr>
<td>Plate Sealing Cover</td>
<td>-</td>
<td>1 pc</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
- Phosphate Buffered Saline (PBS), pH = 7.4
- Test tubes for diluting samples and tubes for conjugates
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Adjustable Precision pipettes to deliver 5-1000 µl with disposable tips
- Multichannel pipette to deliver 25 - 125 µl with disposable tips
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 – 1200 rpm
- Microplate washer suited for magnetic beads washing
- Biocode® 1000A™ detection system and analysis software

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:
96-well Flat Bottom Microtiter Plate
Detection Buffer
Dilution Buffer
Biotin Diluent
Streptavidin – R Phycoerytrin Diluent

Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

- Assay reagents supplied concentrated or lyophilized:

**Antibody Coated Beads**
**Antibody Coated Beads** are supplied as a 50x concentrate and must be diluted prior to use. Vortex the vial for ca. 30 second to break up any bead aggregates. Dilute the concentrated 50x Antibody Coated Beads in PBS to prepare an assay working solution just prior to use. Protect Antibody Coated Beads from light during handling. Example: 120 μl of 50x Antibody Coated Beads + 5880 μl of PBS for use of all 96-well.

**Biotin Labelled Antibody Conjugate**
**Biotin Labelled Antibody** is supplied as a 50x concentrate and must be diluted prior to use. Dilute the concentrated 50x Biotin Labelled Antibody in Dilution Buffer to prepare a 1x working solution just prior to use. Example: 60 μl of 50x Biotin Labelled Antibody + 2 940 μl of Dilution Buffer for use of all 96-well.

**Streptavidin - R Phycoerytrin Conjugate**
**Streptavidin - RPE** is supplied as a 100x concentrate and must be diluted prior to use. Dilute the concentrated 100x Streptavidin - RPE in Dilution Buffer to prepare a 1x working solution just prior to use. Protect Streptavidin - RPE from light during handling. Example: 60 μl of 100x Streptavidin - RPE + 5940 μl of Dilution Buffer for use of all 96-well.

**Human Prostate Cancer Assay 1 Master Standard**
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!! Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human PSP94 is **16 ng/ml** in the stock solution.

Prepare set of standards using Standard Diluent as follows:

<table>
<thead>
<tr>
<th>Volume of Standard</th>
<th>Dilution Buffer</th>
<th>PSP94 Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>-</td>
<td>16 ng/ml</td>
</tr>
<tr>
<td>50 μl of Standard 1</td>
<td>150 μl</td>
<td>4 ng/ml</td>
</tr>
<tr>
<td>50 μl of Standard 2</td>
<td>150 μl</td>
<td>1 ng/ml</td>
</tr>
<tr>
<td>50 μl of Standard 3</td>
<td>150 μl</td>
<td>0.25 ng/ml</td>
</tr>
</tbody>
</table>
Prepared Standards are ready to use, do not dilute them.

Stability and storage:
Standard stock solution (Standard 1) should be aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the diluted Standard solutions.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 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 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 human PSP94 in serum and plasma. Additional sample types may be suitable but have not been validated.

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 sample samples 10x with Dilution Buffer just prior to the assay, e.g. 15 µl of sample + 135 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.

Ask for information at info@biovendor.com if assaying other matrices.

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. Dilute sufficient amount of Antibody Coated beads in PBS and pipet 50 μl of this mixture into the wells to be used. Cover the plate and use orbital shaker closed.

2. Wash the wells 2-times with Wash Solution. In any case and any time of assay do not invert the plate. Do not wash the plate manually without special magnetic handle.

3. Pipet 50 μl of diluted Standards, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the proper wells. See Figure 1 for example of work sheet.

4. Incubate the plate at room temperature (ca. 25°C) for 30 minutes on an orbital microplate shaker, shaking 30 seconds at ca. 1100 rpm (to resuspend beads properly) then continue at ca. 300 rpm.

5. Wash the wells 3-times with Wash Solution.

6. Add 25 μl of Biotin Labelled Antibody into each well.

7. Incubate the plate at room temperature (ca. 25°C) for 30 minutes on an orbital microplate shaker, shaking 30 seconds at ca. 1100 rpm (to resuspend beads properly) then continue at ca. 300 rpm.

8. Wash the wells 3-times with Wash Solution.

9. Add 50 μl of Streptavidin – R Phycoerytrin Conjugate into each well.

10. Incubate the plate at room temperature (ca. 25°C) for 10 minutes, on an orbital microplate shaker, shaking 30 seconds at ca. 1100 rpm (to resuspend beads properly) then continue at ca. 300 rpm.

11. Wash the wells 3-times with Wash Solution.

12. Fill the wells with 125 μl of Detection Buffer.

13. Shake the plate at ca. 1100 rpm for 30 seconds at room temperature on an orbital microplate shaker to resuspend the beads and avoid the beads aggregation.

14. Determine the fluorescence of each well using Biocode® 1000A™ detection system and analyse the sample.

If the plate cannot be read immediately; cover (e.g. aluminium foil) and store the plate in the dark place at 2 to 8 °C. The fluorescence is able be read within 24 hours.
12. CALCULATIONS

Assign appropriate Assay for detection and design the assay layout, standard concentrations and appropriate sample dilution. After last step of assay working procedure, uncover the plate and insert it into the XY platform of the Biocode® 1000A™ instrument and analyse the samples. All the identified wells are then analysed. Concentration of samples is determined from the standard curve. The standard curve is constructed by plotting the mean fluorescence (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the five-parameter algorithm (alternatively, four-parameter or some other algorithms can be used).

Results are reported as concentration of PSP94 (ng/ml) in samples. The measured concentration of samples calculated from the standard curve is automatically multiplied by the dilution factor when it is specified.

It may be observed that some samples show exceed the fluorescence signal of the highest standard. In this case you are intended to use higher dilution for these samples. For reanalyzing do not forget multiply results by appropriate dilution factor.

Figure 1: Example of a work sheet.
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Prostate Cancer Assay 1 are presented in this chapter

- Sensitivity
  Pending data.

- Limit of assay
  Pending data

- Specificity
  Pending data.

- Precision
  Pending data.

- Spiking Recovery
  Pending data.
14. DEFINITION OF THE STANDARD

The recombinant human PSP94 is used as the Standard. The recombinant human PSP94 (AA 1 – 94), produced in E.coli, is 12.01 kDa protein containing 94 amino acid residues of the human PSP94 and 10 extra AA.

15. REFERENCES

References to PSP94:


For more references on this product see our WebPages at www.biovendor.com
## EXPLANATION OF SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
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</tr>
<tr>
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<td><img src="image" alt="storage" /></td>
<td>Storage conditions</td>
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<tr>
<td><img src="image" alt="identification" /></td>
<td>Identification of packaging materials</td>
</tr>
<tr>
<td><img src="image" alt="in vitro" /></td>
<td>In vitro diagnostic medical device</td>
</tr>
</tbody>
</table>
Assay Procedure Summary

- **Prepare Wash Solution**

- **Reconstitute Master Standard** and prepare set of Standards

- **Prepare 96-well Flat Bottom Microtiter Plate**

  - **Insert Beads** into the wells 50 μl

  - **Wash 2x**

  - **Add Standards, blank and samples** 50 μl

  - **Incubate at RT 30 seconds 1100 rpm, 30 minutes 300 rpm/ darkness**

  - **Wash 3x**

  - **Add Biotin Labelled Antibody** 25 μl

  - **Incubate at RT 30 seconds 1100 rpm, 30 minutes 300 rpm/ darkness**

  - **Wash 3x**

  - **Add Streptavidin - R Phycoerytrin Conjugate** 50 μl

  - **Incubate at RT 30 seconds 1100 rpm, 10 minutes 300 rpm/ darkness**

  - **Wash 3x**

  - **Add Detection Buffer** 125 μl

  - **Shake the plate 30 seconds 1100 rpm/ darkness**

  - **Uncover the plate, insert the microplate into the Reader and analyse the samples. Calculate results.**

- **Prepare mixture of Antibody Coated Beads**

- **Dilute samples 10x**

- **Prepare Wash Solution**

- **Reconstitute Master Standard** and prepare set of Standards
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Use only the current version of Product Data Sheet enclosed with the kit!
17. INTENDED USE

The Human Prostate Cancer Assay 2 is a fluorescent bead based immunoassay for the quantitative measurement of human Clusterin. This singleplex protein assay is characterized by high sensitivity and wide dynamic range of the measurement.

18. 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.

19. INTRODUCTION

Clusterin (Apolipoprotein J; SP-40,40; TRPM-2; SGP-2; pADHC-9; CLJ; T64; GP III; XIP8) is a highly conserved disulfide-linked secreted heterodimeric glycoprotein of 75-80 kDa but truncated forms targeted to nucleus have also been identified. The protein is constitutively secreted by a number of cell types including epithelial and neuronal cells and is a major protein in physiological fluids including plasma, milk, urine, cerebrospinal fluid and semen. Due to its wide tissue distribution many diverse physiological functions have been attributed to clusterin including sperm maturation, membrane recycling, lipid transportation, tissue remodeling, complement inhibition and cell-cell or cell-substratum interactions. Moreover, it was proposed, that clusterin functions is as an extra cellular chaperon that stabilizes stressed proteins in a folding-competent state and protein has also been implicated in programmed cell death. Another defining prominent of clusterin is its induction in many severe physiological disturbances states including kidney degenerative diseases, prostate and vesicle carcinogenesis, ovarian cancer and several neurodegenerative conditions (Alzheimer’s disease).

Recent study demonstrate, that serum clusterin level increases significantly in diabetic type II patients and in patients with developing coronary heart disease, or myocardial infarction. These data raise the possibility that elevated clusterin levels in serum may represent a strong indication of vascular damage.

In patients with systemic lupus erythematosus (SLE) was found reduced serum clusterin levels that correlated inversely with disease activity. Lowered clusterin levels could be involved in the pathogenesis of SLE on account of decreased protective effects.

Another interesting observations obtain in rat model suggest that measurement of urinary clusterin levels may be a useful clinical valuable marker for the severity of renal tubular damage. Furthermore, urinary clusterin may also help to differentiate between tubular and glomerular forms of proteinuria.
Clinical use and areas of investigation:
Prostate cancer
Coronary heart diseases
Myocardial infarction
Neurodegenerative diseases
Kidney degenerative disease
Renal tubular damage

20. TEST PRINCIPLE

In the BioVendor Human Prostate Cancer Assay 2, coupled beads are inserted into the wells and immediately washed. Then standards and samples can be pipetted and incubated in microplate wells. After 120 minutes incubation and washing, biotin-labelled anti-human Clusterin antibody is added and incubated for another 60 minutes. After another washing, streptavidin - R phycoerytrin conjugate is added. After 15 minutes incubation and the last washing step, detection buffer is uploaded.
Applied Biocode reader quantifies the fluorescent value of all beads for each bead type in the well. Beads for individual analytes contain specific barcode, which determines the bead identity. The intensity of phycoerythrin fluorescence intensity is proportional to the concentration of each analyte. A standard curve is constructed by plotting fluorescence values against concentrations of standards, concentrations of unknown samples are determined using this standard curve.

21. 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 animal origin. These materials should be handled as potentially infectious
- Streptavidin - R Phycoerytrin Conjugate is light sensitive. Protect it from light to avoid photobleaching of the label
- The materials must not be pipetted by mouth

22. TECHNICAL HINTS
- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware and polypropylene tubes
- 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
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

23. REAGENT SUPPLIED

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>State</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well Flat Bottom Microtiter Plate</td>
<td>ready to use</td>
<td>96 wells</td>
</tr>
<tr>
<td>Human Prostate Cancer Assay 2 Antibody Coated Beads (50x)</td>
<td>concentrated</td>
<td>120 ul/ 1 vial</td>
</tr>
<tr>
<td>Biotin Labelled Antibody Conjugate (50x)</td>
<td>concentrated</td>
<td>60 ul/ 1 vial</td>
</tr>
<tr>
<td>Streptavidin – R Phycoerytrin Conjugate (100x)</td>
<td>concentrated</td>
<td>60 ul/ 1 vial</td>
</tr>
<tr>
<td>Detection Buffer</td>
<td>ready to use</td>
<td>20 ml</td>
</tr>
<tr>
<td>Human Prostate Cancer Assay 2 Master Standard</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>ready to use</td>
<td>50 ml</td>
</tr>
<tr>
<td>Biotin and Streptavidin – R Phycoerytrin Diluent</td>
<td>ready to use</td>
<td>10 ml</td>
</tr>
<tr>
<td>Wash Solution Conc. (10x)</td>
<td>concentrated</td>
<td>100 ml</td>
</tr>
<tr>
<td>Plate Sealing Cover</td>
<td>-</td>
<td>1 pc</td>
</tr>
<tr>
<td>Product Data Sheet + Certificate of Analysis</td>
<td>-</td>
<td>1 pc</td>
</tr>
</tbody>
</table>

24. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Phosphate Buffered Saline (PBS), pH = 7.4
- Test tubes for diluting samples and tubes for conjugates
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Adjustable Precision pipettes to deliver 5-1000 μl with disposable tips
- Multichannel pipette to deliver 25 - 125 μl with disposable tips
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 – 1200 rpm
- Microplate washer suited for magnetic beads washing
- Biocode® 1000A™ detection system and analysis software
25. 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:

  96-well Flat Bottom Microtiter Plate
  Detection Buffer
  Dilution Buffer
  Biotin and Streptavidin – R Phycoerytrin Diluent

  Stability and storage:
  Opened reagents are stable 3 months when stored at 2-8°C.

- Assay reagents supplied concentrated or lyophilized:

  Antibody Coated Beads
  Antibody Coated Beads are supplied as a 50x concentrate and must be diluted prior to use. Vortex the vial for ca. 30 second to break up any bead aggregates. Dilute the concentrated 50x Antibody Coated Beads in PBS to prepare an assay working solution just prior to use. Protect Antibody Coated Beads from light during handling. Example: 120 μl of 50x Antibody Coated Beads + 5880 μl of PBS for use of all 96-well.

  Biotin Labelled Antibody Conjugate
  Biotin Labelled Antibody is supplied as a 50x concentrate and must be diluted prior to use. Dilute the concentrated 50x Biotin Labelled Antibody in Biotin Diluent to prepare a 1x working solution just prior to use. Example: 60 μl of 50x Biotin Labelled Antibody + 2 940 μl of Biotin Diluent for use of all 96-well.

  Streptavidin - R Phycoerytrin Conjugate
  Streptavidin - RPE is supplied as a 100x concentrate and must be diluted prior to use. Dilute the concentrated 100x Streptavidin - RPE in Streptavidin - RPE Diluent to prepare a 1x working solution just prior to use. Protect Streptavidin - RPE from light during handling. Example: 60 μl of 100x Streptavidin - RPE + 5940 μl of Streptavidin – RPE Diluent for use of all 96-well.
Human Prostate Cancer Assay 2 Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!! Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human Clusterin is 160 ng/ml in the stock solution.

Prepare set of standards using Standard Diluent as follows:

<table>
<thead>
<tr>
<th>Volume of Standard</th>
<th>Dilution Buffer</th>
<th>Clusterin Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>-</td>
<td>160 ng/ml</td>
</tr>
<tr>
<td>50 μl of Standard 1</td>
<td>150 μl</td>
<td>40 ng/ml</td>
</tr>
<tr>
<td>50 μl of Standard 2</td>
<td>150 μl</td>
<td>10 ng/ml</td>
</tr>
<tr>
<td>50 μl of Standard 3</td>
<td>150 μl</td>
<td>2.5 ng/ml</td>
</tr>
<tr>
<td>50 μl of Standard 4</td>
<td>150 μl</td>
<td>0.63 ng/ml</td>
</tr>
<tr>
<td>50 μl of Standard 5</td>
<td>150 μl</td>
<td>0.16 ng/ml</td>
</tr>
</tbody>
</table>

Prepared Standards are ready to use, do not dilute them.

**Stability and storage:**
Standard stock solution (Standard 1) should be aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.

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

**Wash Solution Conc. (10x)**
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 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 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

26. PREPARATION OF SAMPLES

The kit measures human Clusterin in serum and plasma. Additional sample types may be suitable but have not been validated.

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 serum samples 3 000x with Dilution Buffer just prior to the assay in two steps as follows: **Dilution A (50x):**
Add 5 µl of sample into 245 µl of Dilution Buffer and mix well (not to foam). Vortex is recommended.

**Dilution B (60x):**
For both measuring in singlets or duplicates add 5 µl of Dilution A into 295 µl of Dilution Buffer to prepare final dilution 3 000x. 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.

Ask for information at info@biovendor.com if assaying other matrices.

*Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results!*

### 27. ASSAY PROCEDURE

15. Dilute sufficient amount of Antibody Coated beads in PBS and pipet 50 µl of this mixture into the wells to be used. Cover the plate and use orbital shaker closed.
16. Wash the wells 2-times with Wash Solution. In any case and any time of assay do not invert the plate. Do not wash the plate manually without special magnetic handle.
17. Pipet 50 µl of diluted Standards, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the proper wells. See Figure 3 for example of work sheet.
18. Incubate the plate at room temperature (ca. 25°C) for **120 minutes** on an orbital microplate shaker, shaking 30 seconds at ca. 1100 rpm (to resuspend beads properly) then continue at ca. 300 rpm.
19. Wash the wells 3-times with Wash Solution.
20. Add 25 µl of Biotin Labelled Antibody into each well.
21. Incubate the plate at room temperature (ca. 25°C) for **60 minutes** on an orbital microplate shaker, shaking 30 seconds at ca. 1100 rpm (to resuspend beads properly) then continue at ca. 300 rpm.
22. Wash the wells 2-times with Wash Solution.
23. Add 50 µl of Streptavidin – R Phycoerytrin Conjugate into each well.
24. Incubate the plate at room temperature (ca. 25°C) for **15 minutes**, on an orbital microplate shaker, shaking 30 seconds at ca. 1100 rpm (to resuspend beads properly) then continue at ca. 300 rpm.
25. Wash the wells 3-times with Wash Solution.
26. Fill the wells with 125 µl of Detection Buffer.
27. Shake the plate at ca. 1100 rpm for **30 seconds** at room temperature on an orbital microplate shaker to resuspend the beads and avoid the beads aggregation.
28. Determine the fluorescence of each well using Biocode® 1000A™ detection system and analyse the sample.

If the plate cannot be read immediately; cover (e.g. aluminium foil) and store the plate in the dark place at 2 to 8 °C. The fluorescence is able be read within 24 hours.

<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>
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<td><strong>A</strong></td>
<td><em>Standard 1</em></td>
<td>Sample 1</td>
<td>Sample 9</td>
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<td><em>Standard 2</em></td>
<td>Sample 2</td>
<td>Sample 10</td>
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<td><em>Standard 3</em></td>
<td>Sample 3</td>
<td>Sample 11</td>
<td>Sample 19</td>
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<td><em>Standard 4</em></td>
<td>Sample 4</td>
<td>Sample 12</td>
<td>Sample 20</td>
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<td>Sample 13</td>
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<td><strong>F</strong></td>
<td><em>Standard 6</em></td>
<td>Sample 6</td>
<td>Sample 14</td>
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<td><strong>G</strong></td>
<td><em>Blank</em></td>
<td>Sample 7</td>
<td>Sample 15</td>
<td>Sample 23</td>
<td>Sample 31</td>
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<td><strong>H</strong></td>
<td><em>Blank</em></td>
<td>Sample 8</td>
<td>Sample 16</td>
<td>Sample 24</td>
<td>Sample 32</td>
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*Figure 3: Example of a work sheet.*

28. **CALCULATIONS**

Assign appropriate Assay for detection and design the assay layout, standard concentrations and appropriate sample dilution. After last step of assay working procedure, uncover the plate and insert it into the XY platform of the Biocode® 1000A™ instrument and analyse the samples. All the identified wells are then analysed. Concentration of samples is determined from the standard curve. The standard curve is constructed by plotting the mean fluorescence (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the five-parameter algorithm (alternatively, four-parameter or some other algorithms can be used).

Results are reported as concentration of Clusterin (ng/ml) in samples. The measured concentration of samples calculated from the standard curve is automatically multiplied by the dilution factor when it is specified.

It may be observed that some samples show exceed the fluorescence signal of the highest standard. In this case you are intended to use higher dilution for these samples. For reanalysing do not forget multiply results by appropriate dilution factor.
29. PERFORMANCE CHARACTERISTICS

Figure 4: Typical Standard Curve for BioVendor Human Prostate Cancer Assay 2.

Typical analytical data of BioVendor Human Prostate Cancer Assay 2 are presented in this chapter.

- Sensitivity
  Pending data.

- Limit of assay
  Pending data

- Specificity
  Pending data.

- Precision
  Pending data.
30. DEFINITION OF THE STANDARD

Standard in this assay is human serum based native clusterin. Native clusterin is 75-80 kDa heterodimeric glycoprotein.

31. REFERENCES

References to Clusterin:


19 Stoop MP, Dekker LJ, Titulaer MK, Burgers PC, Sillevis Smitt PAE, Luider TM, and Hintzen RQ: Multiple sclerosis-related identified in cerebrospinal fluid by advanced mass spectrometry. Proteomics 2008; 8:0000-0000

**For more references on this product see our WebPages at www.biovendor.com**
### EXPLANATION OF SYMBOLS

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<td>See instructions for use</td>
</tr>
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**Assay Procedure Summary**

1. **Prepare Wash Solution**
2. **Reconstitute Master Standard and prepare set of Standards**
3. **Add Standards, blank and samples**
4. **Incubate at RT 30 seconds 1100 rpm, 120 minutes 300 rpm/ darkness**
5. **Wash 3x**
6. **Add Biotin Labelled Antibody**
7. **Incubate at RT 30 seconds 1100 rpm, 60 minutes 300 rpm/ darkness**
8. **Wash 3x**
9. **Add Streptavidin - R Phycoerytrin Conjugate**
10. **Incubate at RT 30 seconds 1100 rpm, 15 minutes 300 rpm/ darkness**
11. **Wash 3x**
12. **Add Detection Buffer**
13. **Shake the plate 30 second 1100 rpm/ darkness**
14. **Uncover the plate, insert the microplate into the Reader and analyse the samples. Calculate results.**