The RD191138300R Human Phosphorylated Neurofilament H ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human phosphorylated neurofilament H (pNF-H).

- It is intended for research use only
- The total assay time is less than 4 hours
- The kit measures pNF-H in serum, plasma and cerebrospinal fluid (CSF)
- Assay format is 96 wells
- Standard and Quality Controls are human brain extract based
- Components of the kit are provided ready to use, concentrated or lyophilized

Clinical application

- Axonal degeneration
- Brain injury
- Neuronal damage
**Immunoassays**

**Test principle**

In the BioVendor Human Phosphorylated Neurofilament H ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with chicken polyclonal anti-pNF-H antibody. After 60 minutes incubation and washing, detection rabbit polyclonal anti-pNF-H antibody is added and incubated with captured pNF-H for 60 minutes. After another washing, HRP conjugated antibody against rabbit antibody 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 pNF-H. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

**Summary of protocol**

- Reconstitute QCs and Master Standard and prepare set of Standards
- Dilute samples 3×
- Add 100 μl Standards, QC and samples
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 μl Detection Antibody Solution
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 μl HRP Conjugate Solution
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 μl Substrate Solution
- Incubate at RT for 15 min
- Add 100 μl Stop Solution
- Read absorbance and calculate results

**HUMAN PHOSPHORYLATED NEUROFILAMENT H ELISA**

**CAT. NO.: RD191138300R**

| Assay format | Sandwich ELISA, HRPlabelled antibody, 96 wells/kit |
| Samples | Cerebrospinal fluid, Plasma, Serum, Tissue extract |
| Controls | QC-Low, QC-High |
| Standards | 62.5 to 4000 pg/ml |
| Limit of detection | 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<sub>blank</sub> + 3SD<sub>blank</sub>) is calculated from the real pNF-H values in wells and is 23.5 pg/ml. *Dilution Buffer is pipetted into blank wells. |

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