# Recombinant Human SPR His-tag

Catalog Number: 10209-SP

## DESCRIPTION

| | |
|---|---|
| **Source** | *E. coli*-derived human SPR protein<br>Met1-Lys261<br>Accession # P35270<br>with a C-terminal 6-His tag |
| **N-terminal Sequence Analysis** | Met1 |
| **Predicted Molecular Mass** | 29 kDa |

## SPECIFICATIONS

| | |
|---|---|
| **SDS-PAGE** | 28 kDa under reducing conditions |
| **Activity** | Measured by its ability to catalyze the reduction of phenanthrenequinone.<br>The specific activity is >700 pmol/min/µg, as measured under the described conditions. |
| **Endotoxin Level** | <0.10 EU per 1 µg of the protein by the LAL method. |
| **Purity** | >90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining. |
| **Formulation** | Supplied as a 0.2 µm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details. |

## Activity Assay Protocol

**Materials**

- Assay Buffer: 50 mM Potassium Phosphate, 150 mM NaCl, pH 6.0
- Recombinant Human SPR His-tag (rhSPR) (Catalog # 10209-SP)
- Substrate: 9,10-Phenanthrenequinone (PQ) (Sigma, Catalog # 156507), 5 mM stock in N,N-Dimethylformamide (DMF)
- β-Nicotinamide adenine dinucleotide phosphate reduced, tetrasodium salt (β-NADPH) (Sigma, Catalog# N7505), 10 mM stock in deionized water
- 96-well clear plate (Catalog # DY990)
- Fluorescent Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

**Assay**

1. Dilute rhSPR to 10 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 200 µM PQ and 800 µM β-NADPH in Assay Buffer.
3. Load 50 µL of 10 µg/mL rhSPR into a plate, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of Substrate Mixture.
4. Read at an absorbance of 340 nm, respectively, in kinetic mode of 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol x -1}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 6270 $M^{-1}cm^{-1}$

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

**Final Assay Conditions**

Per Well:

- rhSPR: 0.5 µg
- PQ: 100 µM
- β-NADPH: 400 µM

## PREPARATION AND STORAGE

**Shipping**

The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage**

**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

## DATA

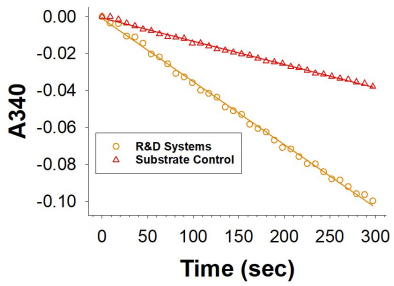


bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

# Recombinant Human SPR His-tag

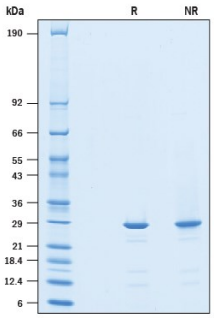
Catalog Number: 10209-SP

**Enzyme Activity**




Recombinant Human SPR His-tag Protein (Catalog # 10209-SP) is measured by its ability to catalyze the reduction of phenanthrenequinone.

**SDS-PAGE**




2 µg/lane of Recombinant Human SPR His-tag was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at 28 kDa under reducing conditions.

## BACKGROUND

Sepiapterin reductase (SPR) catalyzes the biosynthesis of tetra-hydrobiopterin (BH4), an important cofactor in aromatic amino acid metabolism and key regulator of nitric oxide biosynthesis which relates the BH4 cofactor to many pathophysiological processes (1, 2). SPR has additionally been shown to be efficient at mediating chemical redox cycling of quinones and herbicides through a mechanism distinct from sepiapterin reduction (3). SPR is an NADPH-dependent, cytoplasmic enzyme classified as a member of the short-chain dehydrogenase/reductase (SDR) family based on its structural catalytic domain and NADPH binding motifs (4). SPR forms an active homodimer where each monomer contains a key c-terminal asparagine required for activity (3) in addition to the conserved catalytic triad that serves to stabilize protein structure while maintaining cofactor and substrate proximity in a catalytic site composed of several hydrophobic amino acids (4-6). SPR contains the conserved NADPH binding motif at the N-terminus (4-6). SPR has broad tissue expression (7). Identified human mutations in SPR result in compromised production of biopterin cofactor and are associated with neurological deficits (8, 9) linked to dystonia (8, 10). Knockout in mice likewise results in reduced neurotransmitters and movement disorders (11). Loss of BH4 can also reduce nitric oxide production leading to alterations in cardiac function and inflammation (2). SPR has been pharmacologically targeted to reduce pathologically elevated BH4 for pain management (12, 13) and to regulate T-cell function in pathological diseases and tumor growth (14).

**References:**

1. Werner, E.R. *et al.* (2011) Biochem. J **348**:397.
2. Bendall, J. K. *et al.* (2014) Antiox. Redox. Signal **20**:3040.
3. Yang, S. *et al.* (2013) J. Biol. Chem. **288**:19222.
4. Jornvall, H. *et al.* (1995) Biochemistry **34**:6003.
5. Auerbach, G. *et al.* (1997) EMBO J. **16**:7219.
6. Fujimoto, K. *et al.* (2001) Chem. Biol. Interact. **130**:825.
7. Katoh, S. (1971) Arch. Biochem. Biophys. **146**:202.
8. Bonafe, L. *et al.* (2001) Am. J. Hum. Genet **69**:269.
9. Farrugia, R. *et al.* (2007) Mol. Genet. Metab. **90**:277.
10. Abeling, N.G. *et al.* (2006) Mol. Genet. Metab. **89**:116.
11. Yang, S. *et al.* (2006) Am. J. Hum. Genet. **78**:575.
12. Latremoliere, A. *et al.* (2015) Neuron. **86**:1393.
13. Fujita, M. *et al.* (2019) Arthritis Rheumatol. [Epub ahead of print]
14. Cronin, S.J.F. *et al.* (2018) Nature. **563**:7732.



bio-techne®
**Global** bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
**USA** TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
**Europe | Middle East | Africa** TEL +44 (0)1235 529449