Datasheet for ABIN1979382

Sonic Hedgehog ELISA Kit

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Overview

Quantity:	96 tests
Target:	Sonic Hedgehog (SHH)
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Mouse Sonic Hedgehog N-Terminal (Shh-N) ELISA Kit for cell and tissue lysate samples.
Sample Type:	Cell Lysate, Tissue Lysate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes mouse Shh-N.
Cross-Reactivity (Details):	This ELISA kit shows no cross-reactivity with the following cytokines tested: Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin , Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IFN- gamma, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-1beta, IL-2, IL-4, IL-9, IL-10, IL-13, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1alpha, MIP-1gamma, MIP-2, MIP-3beta, MIP-3alpha, PF-4, P-Selectin, RANTES, SCF, SDF-1alpha, TARC, TCA- 3, TECK, TIMP-1, TNF RI, TNF RII, TPO, VCAM-1, VEGF.
Sensitivity:	5 pg/mL
Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection

Product Details

	• Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis • Cell lysate buffer

Target Details

Target:	Sonic Hedgehog (SHH)
Alternative Name:	Shh-N (SHH Products)
Background:	Sonic hedgehog protein (SHH) (HHG-1)
Gene ID:	20423
UniProt:	Q62226
Pathways:	Hedgehog Signaling, Dopaminergic Neurogenesis, Regulation of Muscle Cell Differentiation, Tube Formation, Skeletal Muscle Fiber Development

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well.

3. Incubate 2.5 h at RT or O/N at 4 °C.
4. Add 100 µL of prepared biotin antibody to each well.
5. Incubate 1 h at RT.
6. Add 100 µL of prepared Streptavidin solution to each well.
7. Incubate 45 min at RT.
8. Add 100 µL of TMB One-Step Substrate Reagent to each well.
9. Incubate 30 min at RT.
10. Add 50 µL of Stop Solution to each well.
11. Read at 450 nm immediately.

Reagent Preparation:

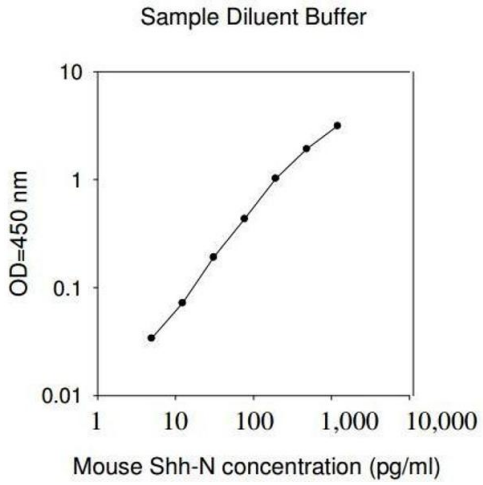
1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: Tissue lysate and cell lysate sample should be diluted at least 5-fold with 1x Sample Diluent Buffer. The Mouse Shh-N ELISA Kit Protocol 3 3. Sample Diluent Buffer (Item D) and Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µL 1x Sample Diluent Buffer (Sample Diluent Buffer should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 12 µL Shh-N standard (50 ng/ml) from the vial of Item C, into a tube with 488 µL Sample Diluent Buffer to prepare a 1,200 pg/ml standard solution. Pipette 300 µL Assay Diluent A or 1x Assay Diluent B into each tube. Use the 1,200 pg/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Sample Diluent Buffer serves as the zero standard (0 pg/ml). 12 µL standard + 488 µL 200 µL 200 µL 200 µL 200 µL 200 µL 200myl 1,200 480 192 76.8 30.72 12.29 4.92 0 pg/ml pg/ml pg/ml pg/ml pg/ml pg/ml pg/ml 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diuent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be The Mouse Shh-N ELISA Kit Protocol 4 diluted 80-fold with 1x Assay Diuent and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 300-fold with 1x Assay Diuent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 40 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1x Assay Diluent to prepare a 300-fold diluted HRP- Streptavidin solution (don't store the diluted solution for next day use). Mix well. 8. Cell lysate buffer should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

Application Details

Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 3. 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 3. 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.
Assay Precision:	Intra-Assay: CV<10% Inter-Assay: CV<12%
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months



ELISA
Image 1.

