ab155450 – Shh-N Mouse ELISA Kit

Instructions for Use

For the quantitative measurement of Shh-N in mouse cell lysates and tissue lysates.

This product is for research use only and is not intended for diagnostic use.

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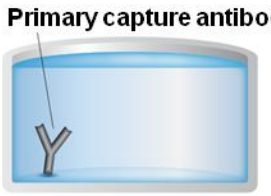
1. BACKGROUND

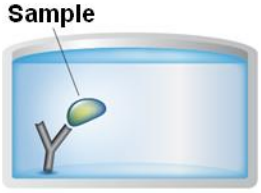
Abcam's Shh-N Mouse ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay designed for the quantitative measurement of mouse Shh-N in cell lysates and tissue lysates.

This assay employs an antibody specific for mouse Shh-N coated on a 96-well plate. Standards and samples are pipetted into the wells and Shh-N present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse Shh-N antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Shh-N bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

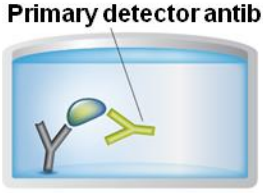
2. ASSAY SUMMARY



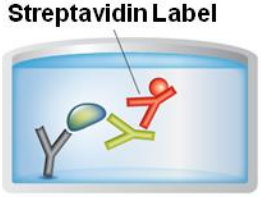
Prepare all reagents, samples and standards as instructed.



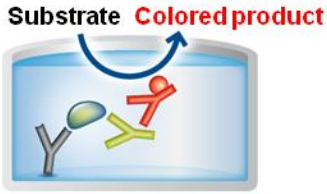
Add standard or sample to each well used. Incubate at room temperature



Add prepared biotin antibody to each well. Incubate at room temperature.



Add prepared Streptavidin solution. Incubate at room temperature.



Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Shh-N Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer concentrate	25 mL	-20°C
Recombinant Mouse Shh-N Standard	2 vials	-20°C
5X Sample Diluent Buffer	10 mL	-20°C
5X Assay Diluent	15 mL	-20°C
Biotinylated anti-mouse Shh-N	2 vials	-20°C
300X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C
2X Cell Lysate Buffer	5 mL	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare standard or sample dilutions.
- Log-log graph paper or computer and software for ELISA data analysis.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps. When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the

vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.

- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 **1X Sample Diluent Buffer**

Dilute 5X Sample Diluent Buffer 5-fold with deionized or distilled water before use.

9.2 **1X Assay Diluent Solution**

Dilute 5X Assay Diluent 5-fold with deionized or distilled water before use.

9.3 **1X Wash Buffer Solution**

If the 20X Wash Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.4 **1X Biotinylated Shh-N Detection Antibody**

Briefly spin the Biotinylated anti-mouse Shh-N vial before use. Add 100 µL of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent E and used in Assay Procedure.

9.5 **1X HRP-Streptavidin Solution**

Briefly spin the 300X HRP-Streptavidin concentrate vial before use. The 300X HRP-Streptavidin concentrate should be diluted 300-fold with 1X Assay Diluent Solution.

For example: Briefly spin the vial of 300X HRP-Streptavidin concentrate 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.

9.6 **1X Cell Lysate Buffer**

Dilute 2X Cell lysate buffer 2-fold with deionized or distilled water before use.

10. STANDARD PREPARATIONS

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.

10.1 Briefly spin the vial of Shh-N Standard. Prepare a 50 ng/mL **Stock Standard** by adding 400 µL 1X Sample Diluent Buffer into the vial (see table below).

10.2 Ensure the powder is thoroughly dissolved by gentle mixing.

10.3 Label tubes #1-8.

10.4 Prepare **Standard #1** by adding 12 µL 50 ng/mL **Stock Standard** to 488 µL 1X Sample Diluent Buffer into tube #1 Mix thoroughly and gently.

10.5 Pipette 300 µL 1X Sample Diluent Buffer into each tube.

10.6 Prepare **Standard #2** by transferring 200 µL from tube #1 to tube #2, mix thoroughly.

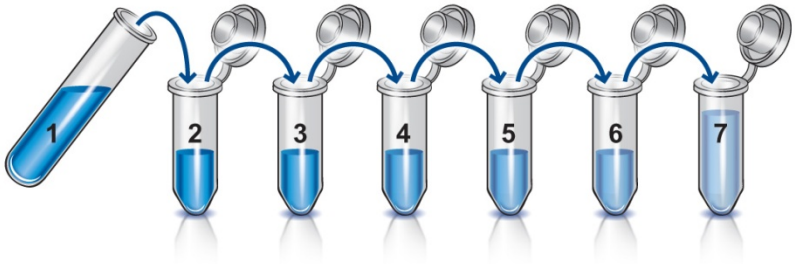
10.7 Prepare **Standard #3** by transferring 200 µL from tube #2 to tube #3, mix thoroughly.

10.8 Using the table below as a guide, prepare further serial dilutions.

10.9 1X Sample Diluent Buffer serves as the zero standard, 0 pg/mL (tube #8).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	12	488	500	50,000	1,200
2	200	300	500	1,200	480
3	200	300	500	480	192
4	200	300	500	192	76.8
5	200	300	500	76.8	30.72
6	200	300	500	30.72	12.29
7	200	300	500	12.29	4.92
8	0	200	200	0	0



11. SAMPLE PREPARATION

General Sample Information:

- Tissue lysate and cell lysate samples should be diluted at least 5-fold with 1X Sample Diluent Buffer.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1. Add 100 µL of each standard (see Standard Preparations, section 10) 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.

13.2. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

13.3. Add 100 µL of 1X prepared biotinylated antibody (see Reagent Preparation, section 9) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4. Discard the solution. Repeat the wash as in step 13.2.

13.5. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation, section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6. Discard the solution. Repeat the wash as in step 13.2.

13.7. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

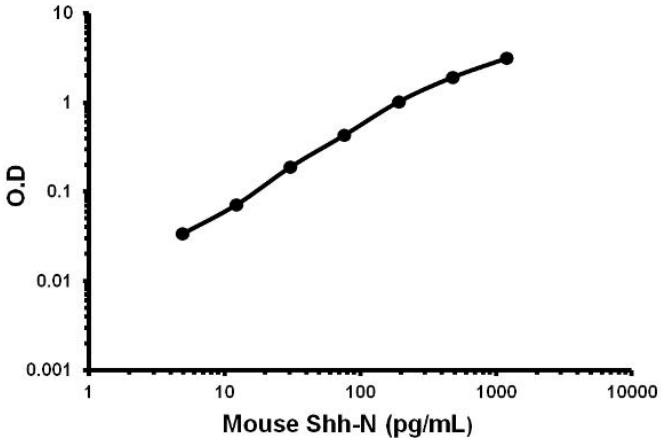
13.8. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. CALCULATIONS

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, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D.
40.96	0.03
102.40	0.07
256	0.18
640	0.38
1,600	0.86
4,000	1.60
10,000	3.00

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of Shh-N is typically less than 5 pg/mL.

RECOVERY –

Recovery was determined by spiking various levels of mouse Shh-N into mouse tissue lysate and cell lysate. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Tissue lysate	130.3	114-150
Cell lysate	126.2	114-137

LINEARITY OF DILUTION -

Tissue Lysate dilution	Average % Expected Value	Range (%)
1:2	74.65	67-81
1:4	89.40	79-99

Cell Lysate dilution	Average % Expected Value	Range (%)
1:2	96.04	90-103
1:4	89.84	84-96

PRECISION –

	Intra-Assay	Inter-Assay
%CV	<10%	<12%

17. ASSAY SPECIFICITY

Cross Reactivity: 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-γ, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 α, IL-1β, 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-1α, MIP-1γ, MIP-2, MIP-3β, MIP-3α, PF-4, P-Selectin, RANTES, SCF, SDF-1α, TARC, TCA-3, TECK, TIMP-1, TNF RI, TNF RII, TPO, VCAM-1, VEGF.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. NOTES

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