AB313941 – HAMSTER (CHO) NIDOGEN-1 ELISA Kit

For the quantitative measurement of Nidogen-1 in Hamster (CHO) culture supernatant and culture extract samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab313941

Storage and Stability:

The entire ELISA 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. Observe the storage conditions for individual prepared components in the Reagent Preparation

Materials Supplied

Item	Quantity	Storage Condition
Nidogen-1 Antibody-coated ELISA Micro Plate	1 unit	2-8ºC
20X Wash Solution Concentrate	50 mL	2-8ºC
Lyophilized Nidogen-1 Calibrator	1 vial	2-8ºC
Biotinylated Nidogen-1 Detection Antibody 100X	1 vial	2-8ºC
100X HRP-Streptavidin Concentrate	150 µL	2-8ºC
Chromogen Substrate Solution	12 mL	2-8ºC
Stop Solution	12 mL	2-8ºC
5X Diluent Concentrate	50 mL	2-8ºC

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

Dilution of Samples

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

• CHO culture supernatant samples – Recommended starting dilution is 1/800. To prepare a 1/800 dilution of a sample, transfer 5 µL of sample to 45 µL of 1X diluent. This gives 1/10 dilution. To prepare 1/80 dilution transfer 5 uL of 1/10 dilution to 395 uL of 1X diluent. This gives you a 1/800 dilution. Mix thoroughly.
• CHO culture extract samples – Recommended starting dilution is 1/80. To prepare a 1/80 dilution of a sample, transfer 5 µL of sample to 395 µL of 1X diluent. This gives 1/80 dilution. Mix thoroughly.

Reagent Preparation

- Equilibrate all reagents to room temperature (16-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

Diluent Concentrate - The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts distilled or deionized water).

Wash Solution Concentrate - The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts distilled or deionized water). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35 ºC before dilution can dissolve crystals.

Biotinylated Nidogen-1 Detection Antibody - Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Detection Antibody to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

HRP-Streptavidin - Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL HRP-Streptavidin to 990 µL of 1X Diluent for each

test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

Pre-coated ELISA Micro Plate - Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

Lyophilised Calibrator preparation :

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well.

1. Add 1.0 mL of distilled or de-ionized water to the Hamster (CHO) Nidogen-1 Calibrator and mix gently until dissolved. The calibrator is now at the concentration stated on the vial.

Note: The reconstituted Hamster (CHO) Nidogen-1 Calibrator should be aliquoted and stored frozen. Avoid multiple freeze-thaw cycles.

2. Label tube numbers 1 - 7.

3. Prepare Standard #1 by adding the appropriate volume of 1X Diluent Solution (see below) to tube #1. Add 60 µL of stock Hamster (CHO) Nidogen-1 Calibrator to obtain a concentration at 20 ng/mL and mix thoroughly and gently.
***Example: Note: This example is for demonstration purposes only. Please remember to check your calibrator vial for the actual concentration of calibrator provided**.

CS = Starting concentration of reconstituted Hamster (CHO) Nidogen-1 Calibrator
CF = Final concentration of Hamster (CHO) Nidogen-1 Calibrator for the assay procedure (20 ng/mL)
VA = Total volume of stock Hamster (CHO) Nidogen-1 Calibrator to dilute (e.g. 85 µL)
VD = Total volume of 1X Diluent Solution required to dilute stock Hamster (CHO) Nidogen-1 Calibrator to prepare Standard #1
VT = Total volume of Standard #1
DF = Dilution factor

Calculate the dilution factor (DF) between stock calibrator & **the Standard #1** final conc.:

Cs/CF = DF

258.333 /20 = 12.91

Calculate the final volume VD required to prepare the Standard #1 at 20 ng/mL

VA * DF = VT

VD = VT - VA

60 * 12.291= 775 µL

VD = 775 – 60 = 715 µL

To tube #1, add 80 µL of reconstituted Hamster (CHO) Nidogen-1 Calibrator to 800 µL of 1X Diluent Solution to obtain a concentration at 20 ng/mL (Standard #1).

4. Add 300 µL 1X Diluent Solution into tube numbers 2 - 6.

5. Prepare Standard #2 by adding 300 µL Standard #1 to tube #2. Mix thoroughly and gently.

6. Prepare Standard #3 by adding 300 µL from Standard #2 to #3. Mix thoroughly and gently.
7. Using the table below as a guide to prepare further serial dilutions. 1X Diluent Solution serves as the zero standard (0 ng/mL)

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc (ng/mL)	Final Conc. (ng/mL)
7	See step 3				20
6	300	300	600	20	10
5	300	300	600	10	5
4	300	300	600	5	2.50
3	300	300	600	2.5	1.25
2	300	300	600	1.25	0.63
1	300	300	600	0.63	0.31

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use. We recommend that you assay all standards, controls and samples in duplicate. Prepare all reagents, working standards, and samples as directed in the previous sections.

1. Label removable 8-well strips as appropriate for your experiment.

2. Add 100 µL of standard into appropriate wells.
   Standard 0 (0.0 ng/mL) in duplicate
   Standard 1 (0.31 ng/mL) in duplicate
   Standard 2 (0.63 ng/mL) in duplicate
   Standard 3 (1.25 ng/mL) in duplicate
   Standard 4 (2.5 ng/mL) in duplicate
   Standard 5 (5 ng/mL) in duplicate

Standard 6 (10 ng/mL) in duplicate
Standard 7 (20 ng/mL) in duplicate

3. Add 100 µL of sample (in duplicate) into pre designated wells. Cover the wells and incubate for 2 hours at room temperature with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multichannel Pipette or auto-washer. Complete removal of liquid at each step is essential for 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.

5. Add 100 µL of 1X prepared biotinylated detection antibody to each well. Incubate for 20 minutes at room temperature with gentle shaking. Keep plate covered in the dark and level during incubation

6. Discard the solution. Repeat the wash as in step 3.

7. Add 100 µL of prepared Streptavidin solution each well. Incubate for 20 minutes at room temperature with gentle shaking. Keep plate covered in the dark and level during incubation

8. Discard the solution. Repeat the wash as in step 3.

9. Add 100 µL of TMB Substrate Reagent to each well. Incubate for 10 minutes at room temperature in the dark with gentle shaking.

10. Add 100 µL of Stop Solution 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.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus