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ab246532
Mouse GDH
SimpleStep ELISA® Kit

For the quantitative measurement of GDH in mouse cell and tissue extracts, serum, and plasma samples.

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

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1. Overview

GDH *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of GDH protein in mouse cell and tissue extracts, serum, and plasma samples.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Mitochondrial glutamate dehydrogenase (GDH) is an enzyme that converts L-glutamate into alpha-ketoglutarate. GDH plays a key role in glutamine anaplerosis by producing alpha-ketoglutarate, an important intermediate in the tricarboxylic acid cycle.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add 50 µL standard or sample to appropriate wells

Add 50 µL Antibody Cocktail to all wells

Incubate at room temperature for 1 hour

Aspirate and wash each well three times with 350 µL 1X Wash Buffer PT

Add 100 µL TMB Development Solution to each well and incubate for 10 minutes.

Add 100 µL Stop Solution and read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Mouse GDH Capture Antibody 10X	600 µL	+4°C
Mouse GDH Detector Antibody 10X	600 µL	+4°C
Mouse GDH Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CPR2	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Sample Diluent NS	12 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

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 is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.

- The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.
- To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.
- 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 to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Cell Extraction Buffer PTR (For cell and tissue extracts only):

Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

9.2 1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

9.3 Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPR2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPR2. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the GDH standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the GDH standard by adding 500 µL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 400 ng/mL **Stock Standard** Solution.

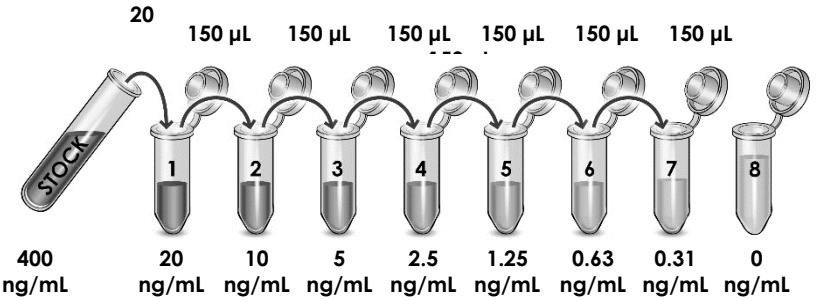
For **cell and tissue extract samples** follow these instructions:

10.1.1 Reconstitute the GDH standard sample by adding 1X Cell Extraction Buffer PTR.

10.1.2 Label eight tubes, Standards 1– 8.

10.1.3 Add 380 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 150 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.

10.1.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



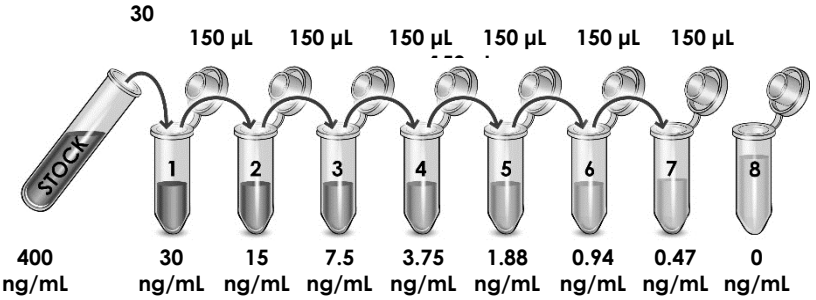
10.2 For **serum and plasma samples** follow these instructions:

10.4.1 Reconstitute the GDH standard sample by adding Sample Diluent NS.

10.2.2 Label eight tubes, Standards 1– 8.

10.2.3 Add 370 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.

10.2.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
NIH/3T3 Cell Extract	15.6 - 250 µg/mL
Liver Tissue Extract	0.6 - 20 µg/mL
Brain Tissue Extract	3.1 - 50 µg/mL
Serum*	≤50%
Plasma – Citrate*	≤50%
Plasma – Heparin*	≤50%

*Based on spiked sample

11.1 Plasma:

Collect plasma using citrate or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

11.3 Preparation of extracts from cell pellets:

11.3.1 Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.

11.3.2 Rinse cells twice with PBS.

11.3.3 Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR.

11.3.4 Incubate on ice for 20 minutes.

11.3.5 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.3.6 Transfer the supernatants into clean tubes and discard the pellets.

11.3.7 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.3.8 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

11.4 Preparation of extracts from tissue homogenates:

11.4.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.4.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

11.4.3 Incubate on ice for 20 minutes.

11.4.4 Centrifuge at 18,000 x g for 20 minutes at 4°C.

11.4.5 Transfer the supernatants into clean tubes and discard the pellets.

11.4.6 Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.4.7 Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

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 immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.

13. 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.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

13.9 Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed

time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Δ **Note**: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

13.10 Analyze the data as described below.

14. Calculations

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

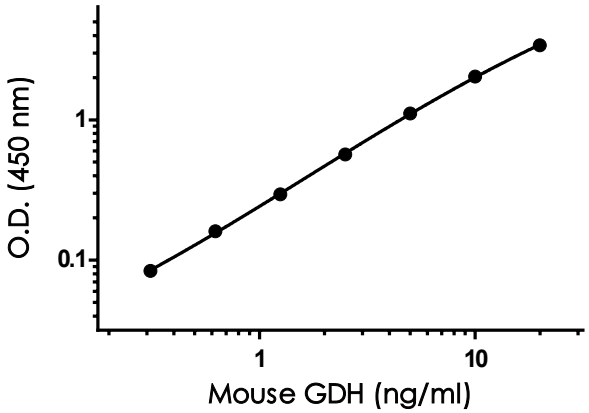
Δ **Note:** Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

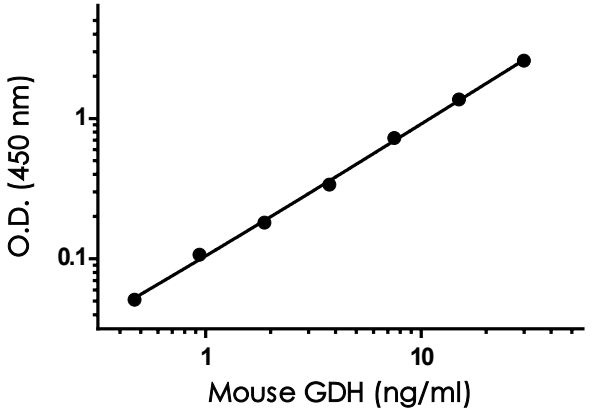
15. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.046	0.054	0.050
0.31	0.135	0.133	0.134
0.63	0.212	0.210	0.211
1.25	0.346	0.344	0.345
2.5	0.629	0.604	0.616
5	1.184	1.138	1.161
10	2.121	2.048	2.085
20	3.441	3.482	3.461

Figure 1. Example of mouse GDH standard curve in 1X Cell Extraction Buffer PTR. The GDH standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
0	0.047	0.056	0.051
0.47	0.102	0.102	0.102
0.94	0.158	0.159	0.159
1.88	0.234	0.23	0.232
3.75	0.394	0.383	0.389
7.5	0.79	0.767	0.778
15	1.431	1.403	1.417
30	2.662	2.610	2.636

Figure 2. Example of mouse GDH standard curve in Sample Diluent NS. The GDH standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. Typical Sample Values

SENSITIVITY –

The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	n=	Minimal Detectable Dose
1X Cell Extraction Buffer PTR	16	60 pg/mL
Sample Diluent NS	19	100 pg/mL

RECOVERY –

Three concentrations of GDH recombinant protein were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
100 µg/mL NIH/3T3 Cell Extract	94	90 - 98
5 µg/mL Liver Tissue Extract	109	104 - 112
25 µg/mL Brain Tissue Extract	111	99 - 116
50% Serum	116	108 - 128
50% Plasma - Citrate	89	87 - 93
50% Plasma - Heparin	98	92 - 102

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native GDH was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	250 µg/mL NIH/3T3 Cell Extract	20 µg/mL Liver Tissue Extract	50 µg/mL Brain Tissue Extract
Undiluted	ng/mL	2.043	11.25	7.122
	% Expected value	100	100	100
2	ng/mL	1.121	4.924	3.363
	% Expected value	110	88	94
4	ng/mL	0.614	2.467	1.502
	% Expected value	120	88	84
8	ng/mL	0.306	1.199	0.779
	% Expected value	120	85	87
16	ng/mL	0.137	0.588	0.378
	% Expected value	107	84	85

Recombinant GDH was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Mouse Serum	50% Mouse Plasma (Citrate)	50% Mouse Plasma (Heparin)
Undiluted	ng/mL	26.09	12.50	25.74
	% Expected value	100	100	100
2	ng/mL	13.23	6.809	14.06
	% Expected value	101	109	109
4	ng/mL	7.113	3.733	7.619
	% Expected value	109	119	118
8	ng/mL	2.972	1.585	3.300
	% Expected value	91	101	103
16	ng/mL	1.379	0.585	1.543
	% Expected value	85	75	96

50% pooled serum and plasma (Heparin and Citrate) samples from healthy donors was measured in duplicate. All values were below the detectable range of the assay.

PRECISION –

Mean coefficient of variations of interpolated values of GDH from two concentrations of mouse liver tissue extract within the working range of the assay.

	Intra-Assay	Inter-Assay
n =	8	3
CV(%)	3.8	3.3

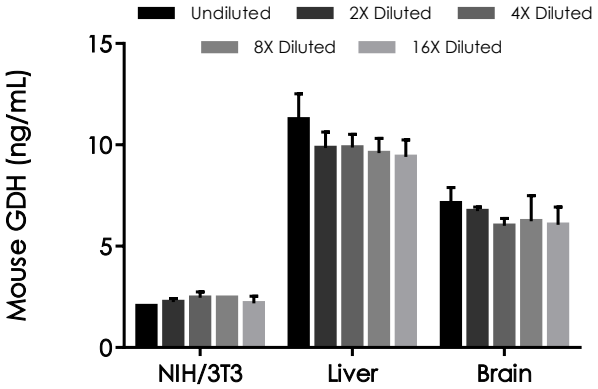


Figure 3. Interpolated concentrations of native GDH in mouse NIH/3T3 cell extract based on a 250 µg/mL extract load, mouse liver tissue extract based on a 20 µg/mL extract load, and mouse brain tissue extract based on a 50 µg/mL extract load. The concentrations of GDH were measured in duplicate and interpolated from the GDH standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean GDH concentration was determined to be 1.940 ng/mL in NIH/3T3 cell extract, 8.822 ng/mL in liver tissue extract, and 5.428 ng/mL in brain tissue extract.

17. Assay Specificity

This kit recognizes both native and recombinant mouse GDH protein in serum, plasma (citrate and heparin), cell and tissue extract samples only.

Urine and cell culture supernatant samples have not been tested with this kit.

This kit is incompatible with plasma (EDTA) samples.

CROSS REACTIVITY

Recombinant mouse GLUD2 protein was prepared at 10 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Recombinant mouse GLUD2 was prepared at 50 ng/mL and 10 ng/mL and tested for interference with 10 ng/mL recombinant mouse GDH protein. No interference with was observed.

18. Species Reactivity

This kit recognizes mouse GDH protein.

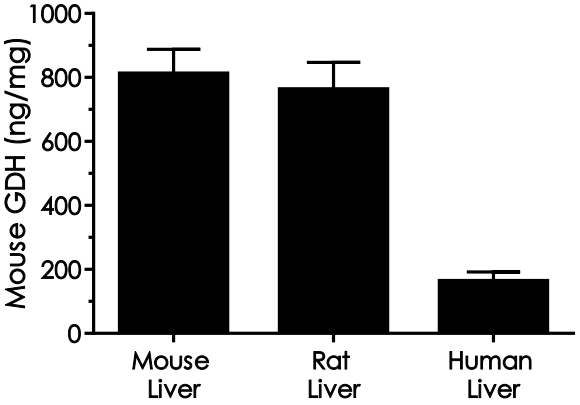


Figure 4. Other species reactivity was determined by measuring liver tissue extract samples of various species, interpolating the protein concentrations from the mouse standard curve, and expressing the interpolated concentrations in ng of GDH protein per mg of extract. The mean GDH concentration was determined to be 811.1 ng/mg in mouse liver extract, 762.4 ng/mg in rat liver extract, and 163.0 ng/mg in human liver extract.

Please contact our Technical Support team for more information.

19. Troubleshooting

Problem	Reason	Solution
Difficulty pipetting lysate; viscous lysate.	Genomic DNA solubilized	Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard 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; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
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 your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB Development Solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.

20. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)