Histidine Assay Kit

ARG83609 Histidine Assay Kit is a detection kit for the quantification of Histidine.

Catalog number: ARG83609

Package: 100 assays

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PRINCIPLE OF THE ASSAY

ARG83609 Histidine Assay Kit t is a sensitive quantitative colorimetric assay for histidine. Histidine Decarboxylase converts histidine to histamine. A Reaction Mix containing WST-1, an electron mediator, and Histamine Dehydrogenase (HDH) is then added. During a brief incubation, the WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. A standard curve is generated from known concentrations of the histidine standard, and samples are then compared to the standard curve to determine the histidine content.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, **10X Assay Buffer and 10X Reaction Buffer** at **RT**, **other component** store at **-80°C**. Use the kit before expiration date.

Component	Quantity	Storage information
L-Histidine Standard (10 mM)	250 µl	-80°C
Histidine Decarboxylase	500 µl	-80°C
Histamine Dehydrogenase	20 µl	-80°C
Probe	1 ml	-80°C
10X Reaction Buffer	30 ml	RT
10X Assay Buffer	30 ml	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate and reader
- Pipettes and pipette tips
- Deionized or distilled water
- Centrifuge spin filter

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid sample.
- Upon receipt, **10X Assay Buffer and 10X Reaction Buffer** at **RT**, **other component** store at **-80°C**. Use the kit before expiration date.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or sample.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Urine- Collect the first urine of the day, micturate directly into a sterile container. Remove impurities by centrifugation. Collect the supernatants and assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Cell or Tissue Lysate- Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect samples and assay immediately or aliquot and store samples at-80°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1x Assay Buffer** - Dilute the **10x Assay Buffer** into Deionized Water to yield **1X Assay Buffer**. The **1x Assay Buffer** is stable for up to 6 months at 2-8°C.
- **1x Reaction Buffer** - Dilute the **10x Reaction Buffer** into Deionized Water to yield **1X Reaction Buffer**. The **1x Reaction Buffer** is stable for up to 6 months at 2-8°C.
- **Working Detection Reagent**- Prepare this reagent immediately prior to use and use it within 20 min after preparation. Probe 1:10 and the Histamine Dehydrogenase 1:1000 in 1X Reaction Buffer.
- **Standards:** Prepare fresh Lysine Standards before use by diluting in 1X Assay Buffer according to the Table below.

Standard tube	Histidine (µM)	1X Assay Buffer (µL)	Standard (µL)
S1	500	475	25 (10 mM Histidine)
S2	250	250	250 of S1
S3	125	250	250 of S2
S4	62.5	250	250 of S3
S5	31.5	250	250 of S4
S6	15.6	250	250 of S5
S7	7.5	250	250 of S6
S0	0	200	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and sample should be assayed in duplicates.

1. Add **50 µl** of **sample** or **each diluted Standard** into respective wells of the 96-well plate.
2. Add **5 µl** of **Histidine Decarboxylase** to **Standard** well.
3. Add **5 µl** of **Histidine Decarboxylase** or **Assay Buffer** to **sample** well.
4. Cover the plate and incubate for **60 minutes** at **37°C**.
5. Add **200 µl** of **Reaction Mix** to each well.
6. Cover the plate and incubate for **15-60 minutes** at **37°C**.
7. Read the absorbance with a plate reader at **O.D. 530-570 nm.**

CALCULATION OF RESULTS

Plot the RFU measured at 60 minutes for each standard against the standard concentrations. Determine the slope using linear regression fitting.

The Histidine concentration of a sample is calculated as follow:

$$\Delta A = A(_{+HDC}) - A(_{-HDC})$$

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Histidine. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.