Histamine Dehydrogenase Activity Assay Kit

ARG83564 Histamine Dehydrogenase Activity Assay Kit can be used to measure Histamine Dehydrogenase in Tissue extracts, Cell lysate, Cell culture media, Other biological fluids

Catalog number: ARG83564

Package: 96 wells

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PRINCIPLE OF THE ASSAY

ARG83564 Histamine Dehydrogenase Activity Assay Kit is a sensitive assay for determining histamine dehydrogenase activity in various samples. Histamine is hydrolyzed by histamine dehydrogenase. The intensity of product color, measured at 450 nm is directly proportional to histamine dehydrogenase activity in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Positive Control at-20 °C and protect from light, all other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard	1 vial (lyophilized)	4 °C
Positive Control	1 vial (lyophilized)	-20 °C
Assay Buffer	4x 30 ml	4 °C
Reaction Buffer	15 ml	4 °C
Substrate	1 vial (lyophilized)	4 °C
Dye Reagent A	1 vial (lyophilized)	4 °C
Dye Reagent B	1 ml	4 °C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Positive Control at-20 °C, all other component at 2-8°C and protect from light. Use the kit before expiration date.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Cell and bacteria- Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection

Tissue- Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

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.

REAGENT PREPARATION

- **Dye Reagent A:** Reconstitute the **Dye Reagent A** with **1 ml** of distilled water. Allow the **Dye Reagent A** keep on bench for few minutes. Make sure the **Dye Reagent A** is dissolved completely and mixed thoroughly before use. The diluted **Dye Reagent A** is stable for 4 weeks at-20°C.

- **Substrate:** Reconstitute the **Substrate** with **1 ml** of Reaction Buffer. Allow the **Substrate** keep on bench for few minutes. Make sure the **Substrate** is dissolved completely and mixed thoroughly before use.
  The diluted **Substrate** is stable for 4 weeks at-20°C.

- **Standard:** Reconstitute the **Standard** with 1 ml of distilled water. Allow the **Standard** keep on bench for few minutes. Make sure the **Standard** is dissolved completely, the concentration will be **1 µmol/ml**. Perform 2-fold serial dilutions of the top standards to make the standard curve.
  The diluted **Standard** is stable for 4 weeks at-20°C.

- **Positive Control:** Reconstitute the **Positive Control** with 0.5 ml of **Assay Buffer**. Allow the **Positive Control** keep on bench for few minutes. Make sure the **Positive Control** is dissolved completely. The diluted **Standard** is stable for 4 weeks at-20°C.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **170 µl** of Substrate into All wells.
2. Add **10 µl** of Sample, Assay Buffer (as **Control**), Positive Control, Standard and Blank into corresponding wells.
3. Add **10 µl** of Dye Reagent A into each wells.
4. Add **10 µl** of Dye Reagent B into each wells.
5. Mix well. Incubate at **RT** for **5 min**. Read the OD at **450nm**.

Summary of Histamine Dehydrogenase Activity Assay Kit Procedure

Reagent	Sample	Control	Positive Control	Standard	Blank
Substrate	170 µl	170 µl	170 µl	170 µl	170 µl
Sample	10 µl	-	-	-	-
Assay Buffer	-	10 µl	-	-	-
Positive Control	-	-	10 µl	-	-
Standard	-	-	-	10 µl	-
Distilled water	-	-	-	-	10 µl
Dye Reagent A	10 µl	10 µl	10 µl	10 µl	10 µl
Dye Reagent B	10 µl	10 µl	10 µl	10 µl	10 µl
Mix well incubate for 5 min at RT Read the OD at 450 nm					

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition:

One unit of HDH activity is defined as the amount of enzyme which reduces 1 µmol of H^+ per min at 37°C.

$C_{Protein}$: the protein concentration, mg/ml;

$C_{Standard}$: the standard concentration, 1 µmol/ml;

W: the weight of sample, g;

V_{Sample}: the volume of sample, 0.01 ml;

$V_{Standard}$: the volume of standard, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 5 minutes.

b.) Calculation:

Formula:

a). According to the volume

$HDH\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times T]$

$= 0.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$

b). According to the weight

$HDH\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{Assay}) \times T]$

$= 0.2 \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times W]$