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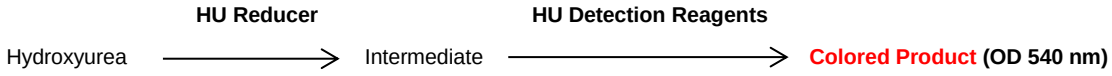
Hydroxyurea Colorimetric Assay Kit

5/20

(Catalog # K2046-100; 100 assays, Store kit at -20°C)

I. Introduction:

Hydroxyurea (HU), also known as carbamohydroxamic acid is a small molecule drug and is used in a number of myeloproliferative, neoplastic, and non-hematological diseases. HU can promote hemoglobin synthesis, reduce thrombocytosis and is an anti-cancer agent for treating chronic myeloid leukemia, melanoma, head and neck cancer and refractory ovarian cancer. HU is also approved by FDA for treating Sickle Cell Disease. Additionally, HU is a ribonucleotide reductase inhibitor and blocks DNA replication and cell division. HU has some side effects including nausea, vomiting, diarrhea, sore throat, ulcers, fever, cough and shortness of breath. Thus, it is important to quantify HU concentration in serum or plasma. **BioVision's Hydroxyurea Colorimetric Assay Kit** is the first, commerically available kit to measure HU concentrations in human serum or plasma. In this assay, HU is reduced by a series of redox reactions to form an intermediate, which in the presence of detection reagents generates a colored product measured at 540 nm. The colored signal is directly proportional to the HU concentration in the sample. The kit offers a simple, rapid, sensitive and convenient way to measure HU concentrations in samples. It can detect as low as 2 nmole of HU under the assay conditions.



II. Application:

- Determination of HU in biological samples

III. Sample Type:

- Serum or plasma

IV. Kit Contents:

Components	K2046-100	Cap Code	Part Number
HU Assay Buffer	2 ml	White	K2046-100-1
HU Standard Solution	20 ml	WM	K2046-100-2
HU Reducer	1 ml	Blue	K2046-100-3
HU Detection I Reagent	10 ml	NM	K2046-100-4
HU Detection II Reagent	10 ml	Amber/NM	K2046-100-5
HU Standard	1 vial	Yellow	K2046-100-6

V. User Supplied Reagents and Equipment:

- **Control Sample** (i..e serum or plasma free of HU)
- Multi-well spectrophotometer
- 96-well clear flat-bottom plate

VI. Storage Conditions and Reagent Preparations:

Store kit at -20°C. The kit components are stable for one year when stored as recommended. Read the entire protocol before performing the experiment.

- **HU Assay Buffer & HU Standard Solution:** Ready to use. Warm at Room Temperature (RT) before use. Store at -20°C.
- **HU Reducer, HU Detection I Reagent & HU Detection II Reagent:** Ready to use. Warm at RT before use. Store at -20°C.
- **HU Standard:** Reconstitute the vial in 1 ml of HU Standard Solution to prepare 12.5 mM HU stock Standard. Store the reconstituted HU stock Standard at -20°C for 2 months.

VII. Hydroxyurea Assay Protocol:

1. **Serum & Plasma Preparation:** Test Serum or plasma samples should be deproteinized using 10 kDa Spin Columns (BioVision Cat. No. 1997). To determine the HU concentration in **Test Sample**(s), a **Control Sample** is required as the reference. Centrifuge the deproteinized Test sample(s) and the Control Sample at 12,000 x *g* and 4°C for 20 min and collect the filtrate for the assay. Add 80 µl of each filtrate type into the respective wells of a 96-well clear plate.
2. **Standard Curve Preparation:** Dilute the 12.5 mM HU stock Standard 100 fold by adding 10 µl HU stock Standard to 990 µl of HU Standard Solution to prepare 0.125 mM HU Standard. Add 0, 16, 32, 48, 64 and 80 µl of the 0.125 mM HU Standard into the desired wells to give 0, 2, 4, 6, 8 and 10 nmole of HU Standard/well respectively. Adjust the final volume of each well to 80 µl with HU Standard Solution.
3. **Reagent Addition:** Add 20 µl of HU Assay Buffer to all the wells including Test Sample(s), Control Sample and HU Standards. Then add 4 µl of HU Reducer to all the wells. Tap the plate gently and wait for 2 min at RT. Add 50 µl of HU Detection I Reagent to all the wells. Tap the plate gently and wait for 1 min at RT. Lastly, add 50 µl of HU Detection II Reagent to all the wells, tap the plate gently and wait for 20 min at RT.

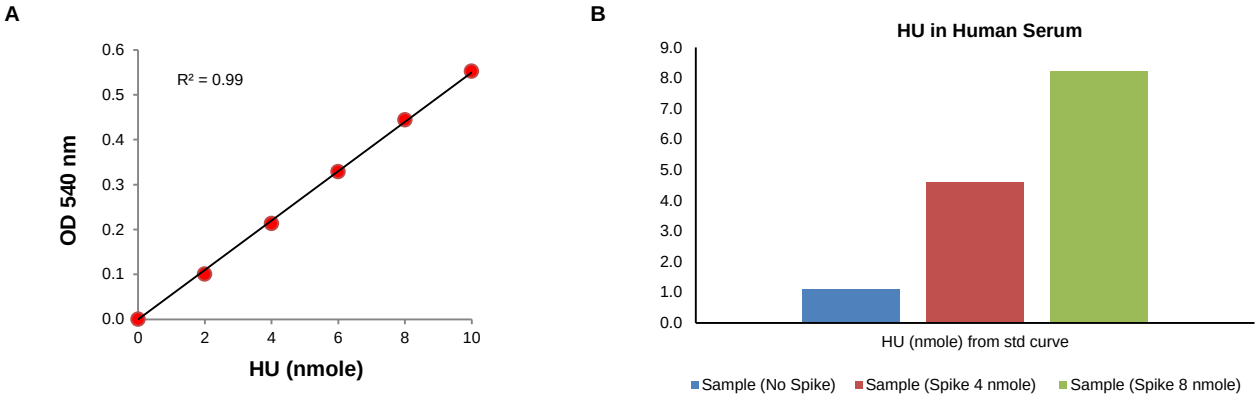
   Notes:

   a. The order of adding the reagents are important.

   b. Do not mix HU Detection I Reagent and HU Detection II Reagent in advance.
4. **Measurement:** Place the 96-well plate into a spectrophotometer and measure the OD at 540 nm in end-point mode at RT.
5. **Calculation:** Subtract the 0 Standard reading from all Standard readings and Control Sample readings from all Test Sample readings respectively. Plot the HU Standard Curve. Apply the corrected Sample readings to the HU Standard Curve to get A nmol of HU formed.

HU concentration (µM) in Sample = A x D/V (nmol/ µl)

Where: A = HU conc from the HU Standard Curve (nmole)
D = Sample dilution factor
V = Volume of sample used (µl)



Figures. A. HU Standard Curve. **B.** HU spiking (4 & 8 nmole) in human serum shows > 85% recovery under the assay kit conditions.

IX. Related Products:

Urea Colorimetric Assay Kit (K375)
Urease Activity Assay Kit (Colorimetric) (K378)
Aspartate Colorimetric/Fluorometric Assay Kit (K552)
Urea Colorimetric Assay Kit II (K376)
Arginine Assay Kit (Fluorometric) (K384)
Citrulline Fluorometric Assay Kit (K2002)

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