Urea Colorimetric Assay Kit

(Catalog #K375-100; 100 assays; Store kit at -20˚C)

I. Introduction:

Urea is a waste product which produced in the liver, dissolved in blood (in a concentration of 2.5 - 7.5 mM), and secreted by the kidneys. Urea also plays a very important role in protein catabolism, removal of toxic ammonia from the body, and the countercurrent system which allows for reabsorption of water and critical ions in the nephrons. Urea determination is very useful for the medical clinician to assess kidney and other organs function of patients. BioVision's Urea Assay Kit provides a rapid, simple, sensitive, and reliable for measurement of Urea level in a variety of samples such as serum, plasma, and urine, etc. In the assay, Urea reacts as substrate with compounds in the presence of enzymes to form a product that reacts with the OxiRed probe to generate color (λ_{max}=570nm). The optical density of produced color has a direct relationship with Urea concentration in the solution. The kit can detect as low as 0.5 nmol per well or 10 µM of Urea. The assay is also suitable for high throughput studies.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Urea Assay Buffer	25ml	WM	K375-100-1
OxiRed Probe in DMSO	200 µl	Red	K375-100-2A
Enzyme Mix (lyophilized)	1 vial	Green	K375-100-3
Developer	1 vial	Orange	K375-100-4
Converting Enzyme	1 vial	Blue	K375-100-5
Urea Standard (100mM)	100 µl	Yellow	K375-100-6

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay. **All the solution in this kit should be kept capped when not being used to prevent absorption of NH_3 from the air.**

IV. Reagent preparation:

Probe: Warm to room temperature before use. Store at -20°C, protect from light and moisture.

Enzyme Mix, Developer and Converting Enzyme: Dissolve in 220 µl Assay Buffer separately. Aliquot to prevent frequent freeze/thaw. Store at -20˚C. Use within two months.

V. Urea Assay Protocol:

1. Standard Curve Preparation:

Dilute the Urea Standard to 0.5 mM by adding 5 µl of the 100 mM Urea Standard to 995 µl dH2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into each well individually. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 1, 2, 3, 4, 5 nmol/well of the Urea Standard.

2. Sample Preparations:

Tissue (20mg) or cells ($2x10^6$) can be homogenized in 100 µl Assay Buffer, centrifuge 15000g for 10 min to remove insoluble materials. 1-25 µl samples (serum, plasma, urine, extracts or other liquid) can be directly added into 96 well plate. Bring the volume to 50 µl/well with Assay Buffer. For unknown samples, we suggest testing several doses of your sample to ensure the readings are within the standard curve range.

Note: Ammonium ion, $NAD^+/NADP^+$, and pyruvate in the sample will interfere with the assay. These compounds can be eliminated by setting a sample control as described in next step.

3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

Sample	Sample Control
42 µl Assay Buffer	44 µl Assay Buffer
2 µl OxiRed Probe	2 µl OxiRed Probe
2 µl Enzyme Mix	2 µl Enzyme Mix
2 µl Developer	2 µl Developer
2 µl Converter Enzyme	

Add 50 µl of the **Reaction Mix** to each well containing the Urea Standard and test samples. Add 50 µl of the Sample Control Mix to sample control well. Mix well. Incubate the reaction for 60 min at 37°C, protect from light.

4. Measurement:

Measure O.D. $_{570nm}$ in a micro plate reader.

5. Calculation:

Subtract 0 nmol standard from all readings. Subtract sample control readings from sample readings. Plot Urea standard Curve. Apply the corrected sample readings to the standard curve. Urea concentrations of the test samples can then be calculated:

$$C = S_a/S_v \text{ nmol/µl or mM}$$

Where S_a is the sample amount of unknown Urea (in nmol) from standard curve.

S_v is sample volume (µl) added into the wells.

Urea Molecular Weight is 60.07g/mol.

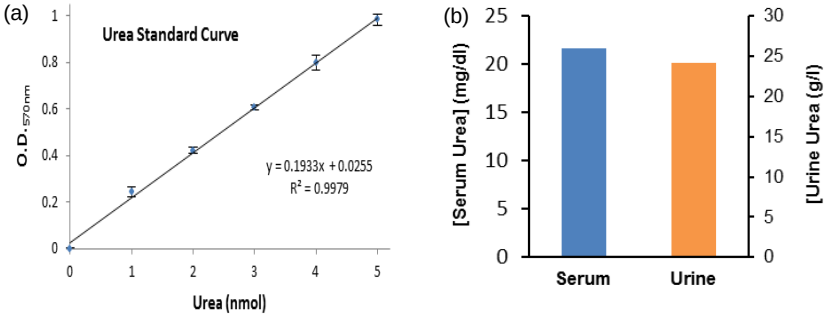

Figure: (a) Urea Standard Curve. (b) Quantitation of urea in human serum (25 µl, 50 times diluted) and urine (25 µl, 5000 times diluted).

RELATED PRODUCTS:

NAD(P)/NAD(P)H Quantification Kit	Phosphate Assay Kits
ADP/ATP Ratio Assay Kit	Ascorbic Acid Quantification Kit
Fatty Acid Assay Kit	Choline/Acetylcholine Quantification Kit
Ethanol Assay Kit	Uric Acid Assay Kit
Pyruvate Assay Kit	Lactate Assay Kit/ II
Ammonia Assay Kit	Triglyceride Assay Kit
Hemin Assay Kit	Glutathione Detection Kit
L-amino Acid Assay Kit	Nitric Oxide Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		