User Instructions

X-Lyz™ Amine Assay Kit

For Total Quantitation of Primary Amines

Catalog # IC286096-Trial

Features:

- ***15min quick assay***
- ***Compatibility with reducing agents***
- ***Wide linear range up to 6000nmol/mL***
- ***Suitable for microplates and cuvettes***

For safely handling the reagents, refer to the MSDS sheets online:
www.x-lyz.com

Contents

1 Product Description

The X-Lyz™ Amine Assay Kit is an amine-reactive reagent for the quantitative determination of total primary amines, such as amino acids (and mixture of AAs), ethanolamine, ammonia, aliphatic and aromatic amines, amino sugars, peptides, and proteins. The water-compatible reagent reacts with the primary amines to form a sensitive chromophore; its absorbance at 350nm is linearly proportional to concentrations over a wide linear range (25-6000nmol/mL). The amine amount is quantified against the standard curve of Norvaline. The method is suitable for assays in microplates and test tubes (cuvettes).

The X-Lyz™ Amine Assay Kit, Catalog # IC286096-Trial, is sufficient for up to 400 assays in microplates and 60 assays in test tubes. The contents are specified in the table below.

Table 1: Contents in X-Lyz™ Amine Assay Kit (Cat No. IC286096-Trial)

Items	Cat No.	Size	Contents	Store	Ship
Reagent A	IC286091	25mL	Containing Reagent A in aqueous solution, pH ~10.5	Room Temp	Room Temp
Reagent B	IC286092	5mL	Containing Reagent B in organic mixture	4ºC	
Norvaline Standard	IC286097	1mL	Containing norvaline 50µmol/mL in 10mM HCl	4ºC	
Instructions	Pub286098	N/A	Instructions for X-Lyz™ Amine Assay Kit	N/A	N/A

All reagents expire one year from the date of purchase.

The assay simply involves mixing the Working Reagent (WR, prepared by combining reagents A and B at a ratio of 5/1) with samples, incubating for 15min before UV measurement at 350nm. The WR/Sample ratio of 10/1 and 1/1 may be used for different concentration ranges. A solid sample can also be analyzed upon dissolution in the WR.

2 Preparation of Standards and Blank

Blank and diluted Norvaline standards will be used to generate a standard curve. Use Table 2 & 3 as a guide to prepare the Blank and diluted Norvaline standards. Starting with the provided Norvaline standard (50µmol/mL, Cat No. IC286097), dilute into the desired concentrations, preferably using the same diluent as the sample(s). The Blank contains only the diluent without Norvaline. The volumes shown in the tables are sufficient for three replications of each dilution.

Table 2: Preparation of Diluted Norvaline Standards for WR/Sample Ratio 10/1 Assays

Vials	Diluent (µL)	Norvaline Sources		Diluted Norvaline Standards	
		Sources	Volume (µL)	Concentration (nmol/mL)	Volume (µL)
1	550	50µmol/mL	75	6000	361
2	264	Vial 1	264	3000	355
3	346	Vial 2	173	1000	360
4	371	Vial 3	159	300	355
5	175	Vial 4	175	150	350
Blank	350	Vial 5	0	0	350

Table 3: Preparation of Diluted Norvaline Standards for WR/Sample Ratio 1/1 Assays

Vials	Diluent (µL)	Norvaline Sources		Diluted Norvaline Standards	
		Sources	Volume (µL)	Concentration (nmol/mL)	Volume (µL)
1	2940	50µmol/mL	60	1000	1600
2	1400	Vial 1	1400	500	1600
3	1200	Vial 2	1200	250	1600
4	1200	Vial 3	800	100	1600
5	1200	Vial 4	400	25	1600
Blank	1600	Vial 5	0	0	1600

Note: To obtain more accurate assay results, the same type of amine standard as the unknown may be used.

3 Preparation of Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

   Total Volume of WR Required = (Blank + # of Standards + # of Unknowns) x (Volume of WR per Replicate) x (# of Replicates) x 1.05 (5% extra)

2. Prepare WR by mixing five parts of Reagent A and one part of Reagent B. Use the WR as soon as possible; sitting too long may cause loss of activity.

For example, to conduct an assay of one blank, five standards and three unknown samples, if 250µL WR is required each in three replicates,

Total Volume of WR Required = (1 + 5 + 3) x 250µL x 3 x 1.05 = 7088µL

A sufficient amount of WR may be prepared by mixing 6mL Reagent A and 1.2mL Reagent B.

4 Assay Procedures

WR/Sample ratio of 10/1 or 1/1 may be used. The 10/1 ratio gives a wider analytical range, while 1/1 is a more sensitive procedure.

Note: For the general estimation of sample concentrations, modern plate readers or spectrophotometers normally have built-in software that automatically plots a linear or nonlinear regression line through the standard points, interpolates the samples, and reports the calculated values. Microsoft Excel can also be used to generate the standard curves, equations and estimate amine samples.

4.1 Microplate Assay Procedure

4.1.1 WR/Sample 10/1 in Microplate

The procedure has a linear range of 150-6000nmol/mL (primary amine concentration)

1. Pipette 25µL of each Blank, standard and sample into a well.
   Note: Different sample sizes may be used as long as the WR/Sample is 10/1. If the sample size is small, e.g. 10µL, it is recommended to use half-area microplates to maintain the detection sensitivity.
2. Add 250µL WR into each well and shake gently. Cover the plate with parafilm.
3. Incubate for at least 15min at temperature between 25-37ºC.
4. Remove parafilm and measure UV absorbance at 350nm in a plate reader.
5. Subtract the average absorbance of the Blank from that of all other standard and unknown sample replicates. The Blank has to be measured every time alongside with the sample(s).
6. Generate a standard curve by plotting the Blank-corrected absorbance of each standard vs. its concentration, and estimate the amine concentration of each unknown sample.

4.1.2 WR/Sample 1/1 in Microplate

The procedure has a linear range of 25-1000nmol/mL (primary amine concentration)

1. Pipette 75µL of each Blank, standard and sample into a well.
2. Add 75µL WR into each well and shake gently. Cover the plate with parafilm.
   Note: Different sample sizes may be used, e.g. 125µL, as long as the WR/Sample is 1/1.
3. Incubate for at least 15min at temperature between 25-37°C.
4. Remove parafilm and measure UV absorbance in a plate reader at 350nm.
5. Subtract the average absorbance of the Blank from that of all other standard and unknown sample replicates. The Blank has to be measured every time alongside with the sample(s).
6. Generate a standard curve by plotting the Blank-corrected absorbance of each standard vs. its concentration, and estimate the amine concentration of each unknown sample.

4.2 Test Tube Assay Procedure

4.2.1 WR/Sample 10/1 in Test Tube

The procedure has a linear range of 150-6000nmol/mL (primary amine concentration)

1. Pipette 0.1mL of each Blank, standard and sample into the test tube or cuvette.
   Note: Different sample sizes may be used, e.g. 0.25mL, as long as the WR/Sample ratio is 10/1.
2. Add 1mL WR into each test tube and gently mix.
3. Incubate for at least 15min at temperature between 25-37°C.
4. Measure UV absorbance 350nm.
   Note: The test sample should be placed in the spectrophotometer chamber right before reading; continuous exposure to UV may affect the absorbance.
5. Subtract the average absorbance of the Blank from that of all other standard and unknown sample replicates. The Blank has to be measured every time alongside with the sample(s).
6. Generate a standard curve by plotting the Blank-corrected absorbance of each standard vs. its concentration, and estimate the amine concentration of each unknown sample.

4.2.2 WR/Sample 1/1 in Test Tube

The procedure has a linear range of 25-1000nmol/mL (primary amine concentration)

1. Pipette 0.5mL of each Blank, standard and sample into the test tube or cuvette.
   Note: Different sample sizes may be used, e.g. 1mL, as long as the WR/Sample ratio is 1/1.

2. Add 0.5mL WR into each test tube and gently mix.
3. Incubate for at least 15min at temperature between 25-37°C.
4. Measure UV absorbance at 350nm.
   Note: The test sample should be placed in the spectrophotometer chamber right before reading; continuous exposure to UV may affect the absorbance.
5. Subtract the average absorbance of the Blank from that of all other standard and unknown sample replicates. The Blank has to be measured every time alongside with the sample(s).
6. Generate a standard curve by plotting the Blank-corrected absorbance of each standard vs. its concentration, and estimate the amine concentration of each unknown sample.

5 Compatibility

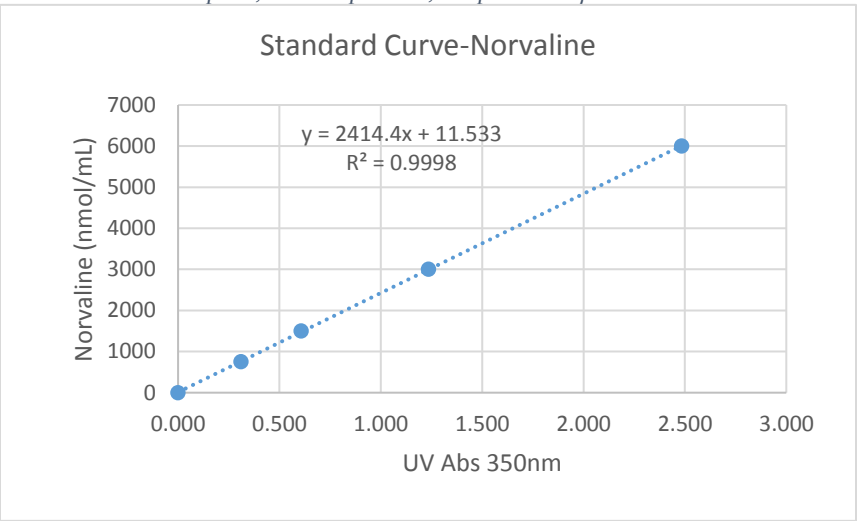
The X-Lyz™ Amine Assay is compatible with reducing agents, common detergents, alcohols, urea, guanidine, metal chelators, and inorganic salts. When high concentrations of these substances are present, however, it is recommended to include them in the standards and Blank.

Non-primary amine buffers may be used to replace TRIS, such as HEPES, BICINE, BIS-TRIS, BIS-TRIS propane, and BIS-TRIS methane.

6 Estimation of Concentration

To estimate sample concentrations, a standard curve is first plotted after measurement; the amine concentrations of unknown samples are then calculated from it. In Figure 1, for example, the standard curve of Norvaline is generated and the linear equation Y = 2414.4X + 11.533 is obtained, where Y = amine concentrations and X = absorbance at 350nm. If an unknown sample, e.g., containing a mixture of amino acids, gives an absorbance of 1.000 (X), the sample's total amine concentration (Y) is estimated be 2426nmol/mL (Y = 2414.4 x 1.000 + 11.533 = 2426nmol/mL).

Figure 1: Standard Curve of Norvaline Concentration vs. Absorbance. The assay was conducted in 96-well plate, WR/Sample 10/1, sample size 25µL.



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