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ab185435

Tyrosine Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Tyrosine in biological samples.

View kit datasheet: www.abcam.com/ab185435
(use www.abcam.cn/ab185435 for China, or www.abcam.co.jp/ab185435 for Japan)

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 3a, Last Updated 11 August 2023

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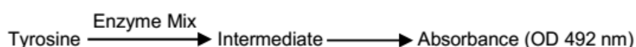
1. Overview

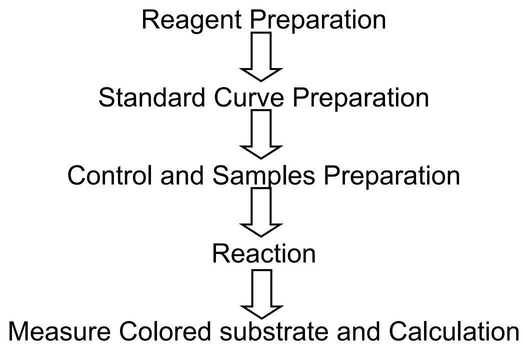
Tyrosine (Tyr) is one of the four standard amino acids containing an aromatic group as a side chain. Its hydrophobicity is one of the main characteristics of this uncharged polar amino acid. In addition to being an essential amino acid Tyr is important in number of biological processes such as the synthesis of neurotransmitters, thyroid hormones, melanine, fumarate and acetoacetate. The pathology of abnormal concentrations of Tyr is well known in diseases including phenylketonuria, hypothyroidism, tyrosinemia, albinism, and alkaptonuria.

Abcam's Tyrosine Assay Kit (Colorimetric) (ab185435) is a simple, yet sensitive assay that is able to detect normal and abnormal concentrations of Tyrosine in biological fluids. The assay is based on the enzymatic oxidation of Tyrosine producing a stable signal (OD 492 nm), which is directly proportional to the amount of Tyrosine. Sample preparation is minimal and does not require strenuous or complicated procedures. The assay can detect as low as 50 µM of Tyrosine in a variety of biological samples.

Figure 1: Assay Procedure



2. Protocol Summary



3. Kits Components

Item	Quantity
Assay Buffer V/Tyr Assay Buffer	25 mL
Tyrosine Enzyme Mix/Tyr Enzyme Mix	1 vial
Tyorsine/Tyr Standard	1 vial

4. Storage and Stability

Upon arrival, store the kit at -20°C and protect from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- Distilled water (dH_2O)
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Centrifuge with cooling option
- 10kDa spin column (ab93349)

6. Reagents Preparation

1. **Assay Buffer V/Tyrosine Assay Buffer:**
   Ready to use as supplied. Aliquot and store at +4°C or -20°C. Warm to room temperature before use.

2. **Tyrosine Enzyme Mix:**
   Reconstitute with 220 µL Assay Buffer V/Tyrosine Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months.

3. **Tyrosine/Tyrosine Standard**
   Reconstitute with 100 µL Assay Buffer V/Tyrosine Assay Buffer to generate 100 mM solution. Store at 4°C. Stable for two months.

7. Assay Protocol

1. Sample Preparation

a. Samples should be deproteinized using 10 kDa Spin Column (ab93349).

b. Briefly, add sample to the spin column, centrifuge at 10,000 x *g* for 10 minutes at 4°C. Collect the filtrate.

c. Add 80-135 µL of filtrate into desired well(s) in 96-well plate.

d. Adjust the volume to 150 µL/well with dH_2O.

NOTE:

- *Tyrosine concentrations can vary over a wide range. Normal ranges in Humans are 55-147 µM for serum, 10-290 µM for urine and 34-112 µM in plasma. Abnormal tyrosine levels can exceed 1.5 mM in tyrosinemia samples. For unknown samples, we recommend doing pilot experiment and testing several doses to ensure the readings are within the Standard Curve range.*
- *Endogenous compounds may interfere with the assay. To ensure accurate determination of Tyr in the test samples or for samples having low concentration of Tyr, we recommend spiking samples with a known amount of Tyrosine/Tyr Standard (30 nmol).*

- *For samples having background, prepare parallel sample well(s) as background control(s).*

2. Standard Curve Preparation:

a) Dilute the Tyrosine/Tyrosine Standard to 2.5 mM by adding 25 µL of 100 mM Tyrosine/Tyrosine Standard to 975 µL of dH_2O.

b) Add 0, 2, 6, 12, 18, 24 and 30 µL of Tyrosine/Tyrosine Standard into series of wells in a 96-well plate to generate 0, 5, 15, 30, 45, 60, and 75 nmol/well of Tyrosine/Tyrosine Standard.

c) Adjust the volume to 150 µL/well with dH_2O. The diluted Standard can be stored at 4°C for subsequent assays.

3. Reaction Mix:

Prepare enough Reaction Mix for the number of assays to be performed. For each well, prepare 50 µL Mix containing:

	Reaction Mix	*Background Control Mix
Assay Buffer V/Tyrosine Assay Buffer	48 µL	50 µL
Tyrosine Enzyme Mix	2 µL	--

Add 50 µL of the reaction mix to each well containing the Standards and samples.

*For samples having high background, and 50 µl of Background Control mix to sample background control well(s). Mix well.

4. Measurement

a) Incubate the plate at room temperature for 60 minutes protected from light.

b) Measure absorbance (OD 492 nm) in a microplate reader.

8. Data Analysis

Calculations:

a) Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the Tyr Standard Curve. For unspiked samples, apply the corrected OD to the Tyr Standard Curve to get B nmol of Tyr in the sample well.

Sample Tyr concentration (C) = B/V X D nmol/ml or µM

Where:

B is the amount of Tyr in the sample well from the standard curve (nmol)

V is the sample volume added into the reaction well (ml)

D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, Tyr amount in sample well (B)

$$\left(\frac{(OD_{\text{sample (corrected)}})}{(OD_{\text{sample + Tyr Std(corrected)}})-(OD_{\text{sample(corrected)}})}\right) * Tyr\ Spike(\text{nmol})$$

Tyrosine molecular weight: 181.2 g/mol.

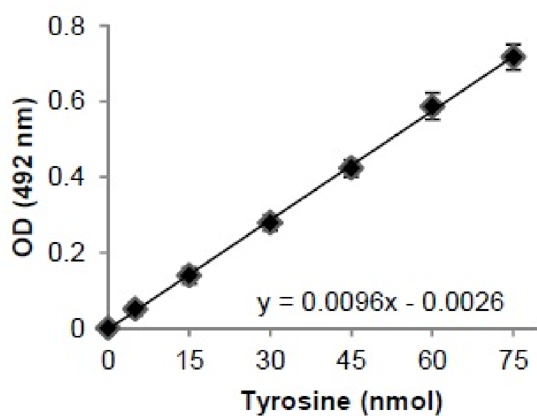


Figure 2. Tyrosine Standard Curve.

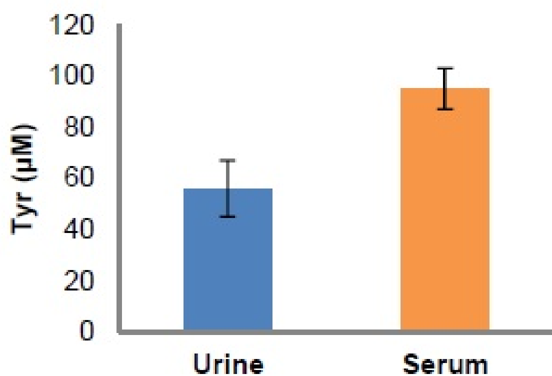


Figure 3. Measurement of Tyrosine concentration in Human urine and serum (135 µL, each). Both samples were deproteinized using 10 kDa Spin Column (ab93349) and spiked with known amount of Tyrosine (30 nmol). Assays were performed following the kit protocol.

9. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature

Problem	Reason	Solution
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

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