

**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](http://www.abcam.com/ab185435)  
(use [www.abcam.cn/ab185435](http://www.abcam.cn/ab185435) for China, or [www.abcam.co.jp/ab185435](http://www.abcam.co.jp/ab185435) for Japan)

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

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# 1. Overview

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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.

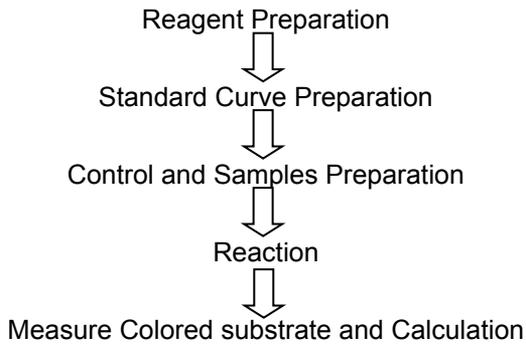
Abcam's Tyrosine Assay Kit (Colorimetric) (ab185435) is a simple, yet sensitive assay that is able to detect normal and abnormal concentrations of Tyrosine in biological fluids. The assay is based on the enzymatic oxidation of Tyrosine producing a stable signal (OD 492 nm), which is directly proportional 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  $\mu\text{M}$  of Tyrosine in a variety of biological samples.

## Figure 1: Assay Procedure



## 2. Protocol Summary

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### 3. Kits Components

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<b>Item</b>	<b>Quantity</b>
Assay Buffer 5	25 mL
Tyrosine Enzyme Mix	1 vial
Tyrosine	1 vial

PLEASE NOTE: [Assay Buffer 5] was previously labelled as [Assay Buffer V] and [Tyr Assay Buffer], and [Tyrosine Enzyme Mix] as [Tyr Enzyme Mix], and [Tyrosine] as [Tyr Standard]. The composition has not changed.

## **4. Storage and Stability**

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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**

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- Distilled water (dH<sub>2</sub>O)
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Centrifuge with cooling option
- 10kDa spin column (ab93349)

## 6. Reagents Preparation

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### 1. Assay Buffer 5:

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  $\mu\text{L}$  Assay Buffer 5. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months.

### 3. Tyrosine

Reconstitute with 100  $\mu\text{L}$   $\text{dH}_2\text{O}$  to generate 100 mM solution.  
Store at 4°C. Stable for two months.

## 7. Assay Protocol

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### 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  $\mu\text{L}$  of filtrate into desired well(s) in 96-well plate.
- d. Adjust the volume to 150  $\mu\text{L}$ /well with  $\text{dH}_2\text{O}$ .

#### NOTE:

- *Tyrosine concentrations can vary over a wide range. Normal ranges in Humans are 55-147  $\mu\text{M}$  for serum, 10-290  $\mu\text{M}$  for urine and 34-112  $\mu\text{M}$  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 (30 nmol).*

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

## **2. Standard Curve Preparation:**

- a) Dilute the Tyrosine to 2.5 mM by adding 25  $\mu\text{L}$  of 100 mM Tyrosine to 975  $\mu\text{L}$  of  $\text{dH}_2\text{O}$ .
- b) Add 0, 2, 6, 12, 18, 24 and 30  $\mu\text{L}$  of Tyrosine into series of wells in a 96-well plate to generate 0, 5, 15, 30, 45, 60, and 75 nmol/well of Tyrosine.
- c) Adjust the volume to 150  $\mu\text{L}$ /well with Assay Buffer 5. 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  $\mu$ L Mix containing:

	Reaction Mix	*Background Control Mix
Assay Buffer 5	48 $\mu$ L	50 $\mu$ L
Tyrosine Enzyme Mix	2 $\mu$ L	--

Add 50  $\mu$ L of the reaction mix to each well containing the Standards and samples.

\*For samples having high background, add 50  $\mu$ L 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

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### 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 Tyrosine Standard Curve. For unspiked samples, apply the corrected OD to the Tyrosine Standard Curve to get B nmol of Tyr in the sample well.

$$\text{Sample Tyr concentration (C)} = B/V \times D \text{ nmol/ml or } \mu\text{M}$$

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)

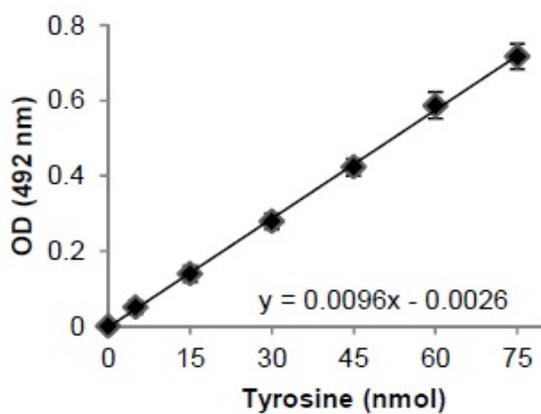
**D** is the sample dilution factor

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

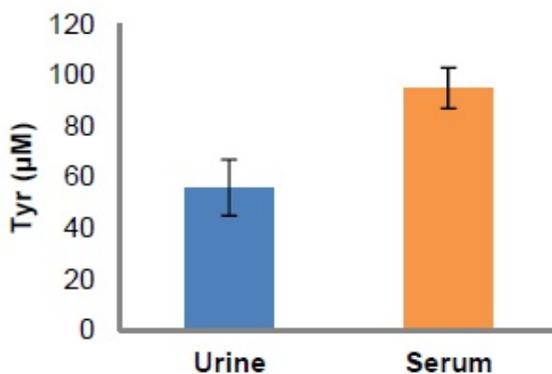
**For spiked samples, Tyr amount in sample well (B)**

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

Tyrosine molecular weight: 181.2 g/mol.



**Figure 2.** Tyrosine Standard Curve.



**Figure 3.** Measurement of Tyrosine concentration in Human urine and serum (135  $\mu$ L, 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

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<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
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

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
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 <b>10kDa spin column (ab93349)</b>
	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

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
	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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