Imidazole ELISA Kit

Catalog Number KA1421

96 assays

Version: 14

Intended for research use only

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Introduction

Intended Use

For the determination of Imidazole in Aqueous Samples.

Background

Imidazole is incorporated into many important biological molecules. The most obvious is the amino acid histidine, which has an Imidazole side chain. One of the applications of Imidazole is in the purification of His-tagged proteins in immobilized metal affinity chromatography (IMAC). Imidazole is used to elute tagged proteins bound to Ni ions attached to the surface of beads in the chromatography column. An excess of Imidazole is passed through the column, which displaces the His-tag from nickel co-ordination, freeing the His-tagged proteins. The Imidazole ELISA kit can be used to determine quantitatively the concentration of Imidazole in the aqueous sample.

Principle of the Assay

The enzyme immunoassay for Imidazole is based on the competition between the Imidazole to be assayed and the Imidazole-alkaline phosphatase conjugate, for binding to antibody directed against Imidazole, coated onto microtiter wells. The sample containing the Imidazole, and the Imidazole-alkaline phosphatase conjugate, when added to the microtiter wells, compete for binding to a limiting number of antibody sites. After incubation, each well is rinsed in order to remove non-bound components. The bound enzymatic activity then is measured by the addition of a chromogenic substrate. The intensity of the color developed is inversely proportional to the concentration of Imidazole in the sample. The concentration is estimated by comparison with standard Imidazole solution.

General Information

Materials Supplied

List of component

Component	Amount
96-wells microtiter plate (#S). Twelve strips of 8 detachable wells, coated with Anti-Imidazole antibody.	96 (8x12) wells
Calibrator containing 0, 1.0, 3.0 and 9.0 µg/mL of imidazole.	0.9 mL x 4
Imidazole-Alkaline Phosphatase conjugate (#3) (IDZ-ALP).	10.5 mL
p-Nitrophenyl Phosphate (pNPP) substrate (#5). Ready to use.	10.5 mL
Wash Buffer (10xPBS-Tween) (#6). Dilute 10 fold with distilled or deionized water to 150 mL prior to use.	15 mL
Stop Solution (#7), 3 N NaOH.	5.5 mL

Storage Instruction

All reagents of the kit are stable, if stored at 2-8°C, until the expiration date stated on the kit.

Materials Required but Not Supplied

- ✓ Pipettors capable of delivering 50 µL and 100 µL.
- ✓ Microtiter plate reader (wavelength 405 nm).
- ✓ Plate washer or squeezable wash bottle.
- ✓ Timer.
- ✓ Absorbent paper towels.

Precautions for Use

- ✓ Reagents are for *in vitro* research use only.
- ✓ Do not mix reagents from different lots.
- ✓ If concentrations of imidazole in the samples are high (>10 µg/mL), dilute the samples with 0.05 M Tris-HCl Buffer, pH 6.5 such that points fall in the middle range of the standard curve.
- ✓ Do not return unused reagents back into their original bottles.
- ✓ Samples tested should have a pH of 7.0 (± 1.0). Excessive alkaline or acidic conditions may affect the test results.
- ✓ The stop solution contains NaOH. Avoid contact with skin or eyes. If exposed, flush with water.
- ✓ Dispose of all materials, containers and devices in the appropriate receptacle after use.

Assay Protocol

Assay Procedure

Let the components of the kit equilibrate to room temperature before use.

1. Carefully add 50 µL of standard or sample (dilute if Imidazole concentration is high) to the bottom of each well. Slightly tap the side of the strip holder to evenly distribute the sample.
2. Avoid touching the well with the pipette tip while adding 100 µL of IDZ-ALP conjugate (#3) to each well. Slightly tap the side of the strip holder to properly mix the sample and enzyme conjugate.
3. Incubate at room temperature for 40 minutes.
4. After incubation, dispose of the solution in the wells by inverting and shaking. Wash microtiter wells 3 times with wash buffer to remove the non-bound conjugate. Washing may be done manually as follows: use squeeze bottle to fill wells gently with wash buffer, dumping the wells between each wash by inverting and shaking. After the third wash, tamp holder with washed strips onto a piece of absorbent paper.
5. Add 100 µL of pNPP substrate (#5) to each well and incubate at room temperature for 20 min. To avoid contamination, place the needed amount of substrate into a test tube and only dispense from the tube itself.
6. Add 50 µL of Stop Solution (#7) to each well and tap the strip holder for proper mixing.
7. Read absorbance at 405 nm using ELISA reader.

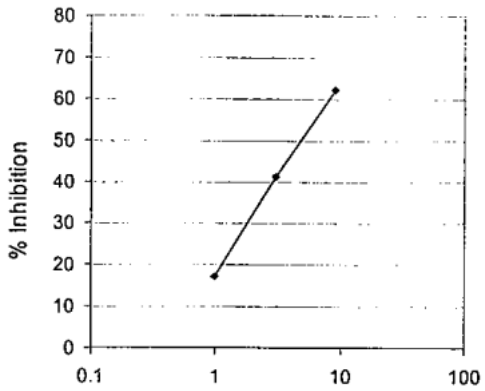
✓ Simplified Assay Procedure

1. Add sample or standard (50 µL).
2. Add Enzyme conjugate (100 µL). 40 min at RT.
3. Wash 3x.
4. Add pNPP (100 µL), wait for 20 min at RT.
5. Add stopping solution (50 µL) and read at 405 nm.

Data Analysis

Calculation of Results

1. Calculation
   a. Average the absorbance (ODs) for each standard concentration of Imidazole including 0 µg/mL (OD_0).
   b. % of Inhibition = 100 - (ODs / OD_0) x 100
2. Plot values of % of Inhibition, step 1 (b), against their corresponding concentrations on Log_{10} paper.
3. Calculate the imidazole concentration in the sample by interpolation and multiply by the sample's dilution factor to obtain the actual quantity of imidazole.



Performance Characteristics

✓ Cross reactivity

Compound	Conc. (µg/mL)	% Inhibition
Histamine	5	42
Histidine	1000	<5
Serotonin	1000	<5
Spermidine	500	<10
Putrescine	500	<10
Spermine	500	<10
Tyramine	500	<10
Cadaverine	500	<10

There is the possibility that other substances and/or factors not listed above may interfere with the test.

Resources

Plate Layout

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3								
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1								
	A	B	C	D	E	F	G	H