Tribioscience

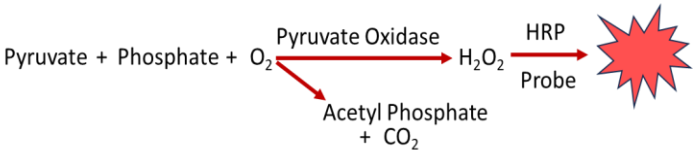
Pyruvate Fluorometric Assay Kit (TBS2023F, 100 assays; store at -20°C)

DESCRIPTION

Pyruvate is a central molecule in metabolic pathways. Pyruvate can be converted to carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, to the amino acid alanine and to ethanol. Abnormal levels of pyruvate have been linked to liver diseases and metabolic disorders.

Tribioscience's Pyruvate Fluorometric Assay kit is designed to be a robust, simple, and high throughput method in which pyruvate is oxidized to generate hydrogen peroxide. The resulting hydrogen peroxide is catalyzed and reacts with the probe measured by the fluorometric method at Ex/Em=530/590 nm.

ASSAY PRINCIPLE



APPLICATIONS

Direct Assays: detect as low as 1 µM of pyruvate in cells, serum and other biological samples.

KIT CONTENTS

Name	Size (100 tests)
Pyruvate standard (125 µM)	200 µL
Enzyme Mix (5X)	1 mL
Pyruvate assay buffer	10 mL
Pyruvate Probe	60 µL

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of 12 months.

ASSAY PROTOCOL

Except Enzyme, warm all the components to room temperature before use. Briefly centrifuge all small vials prior to opening.

1. Sample Preparation:

Tissue (1-10 mg) or cells (1 x 10^6) can be lyses in 100 µL of Assay Buffer. Centrifuge ice cold at 15,000 x g for 2 minutes to pellet insoluble materials and collect supernatant. For more accurate assays, the sample should be quickly frozen using liquid N_2 or dry ice if it is to be assayed later.

2. Standard Curve Preparations:

Tubes	Pyruvate (µL)	Buffer (µL)	Pyruvate Conc (µM)
1	100 µL stock	300	31.25
2	200 µL of Tube#1	200 µL	15.6
3	200 µL of Tube#2	200 µL	7.8
4	200 µL of Tube#3	200 µL	3.9
5	200 µL of Tube#4	200 µL	1.95
6	200 µL of Tube#5	200 µL	0.98
7	0	200 µL	0

Add 50 µL/well standards and samples to 96-well plate, use black plate for fluorometric assay.

3. Pyruvate Reaction Mix: Prepare enough mix for 100 tests as the table:

Pyruvate Reaction Mix	Volume
Pyruvate assay buffer	3.95 mL
Enzyme Mix (5X)	1 mL
Pyruvate Probe	50 µL

Add 50 µL of the Pyruvate Reaction Mix to each well containing the Pyruvate Standards and test samples. Tap plate lightly to mix.

4. Incubation: Incubate at 37ºC for 30 minutes with gentle shaking and protect from light.

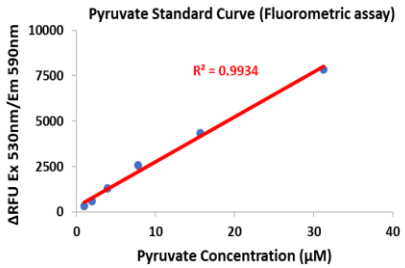
5. Measurement: Measure at Ex/Em = 530/590 nm for fluorometric assay.

6. Calculation: Correct background by subtracting the value of the 0 Pyruvate standard (blank) from all standard readings. Plot the value against standard concentration. Determine the slope using linear regression fitting.

Typical standard curve is Y=AX+B, A is the slope and B is the y-intercept. X is concentration.

Pyruvate = N* [(RFU_{sample}-RFU_{blank})-B] /A (µM)

RFU_{sample} and RFU_{blank} are fluorescence values of the sample and the buffer. **(Note:** If unknown sample results over standard curve range, dilute sample with assay buffer. Repeat the assay; multiply the results by the dilution factor ***N***.)



RELATED PRODUCTS

Pyruvate Colorimetric Assay (TBS2023C)
Glycerol Colorimetric Assay (TBS2204C)
Glycerol Fluorometric Assay (TBS2204F)
Triglyceride Colorimetric Assay (TBS2205C)
Triglyceride Fluorometric Assay (TBS2205F)
LDH Cytotoxicity Assay (TBS2002)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay (TBS2020)
Cell Count Kit -8 (TBS2022)
XTT Cell Viability Assay (TBS2021)
Caspase-3 Colorimetric Assay (TBS2030)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
NNMT Inhibitor Screening Assay (TBS2097)
NNMT Activity Fluorometric Assay (TBS2098)
G6PDH Activity Colorimetric Assay (TBS2102
Cytochrome c Reductase Activity Assay (TBS2116)

FOR RESEARCH ONLY

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