CATACHEM

PYRUVATE
MANUAL OR AUTOMATED ASSAY
Cat. No. C750-0A (6 x 25 mL)

Intended Use
For **In Vitro Diagnostic** use in the quantitative determination of Pyruvate in serum or plasma.

Clinical Significance (1)
Pyruvate is one of main intermediates in the metabolic pathway. It can be converted into energy through acetyl-CoA, to a carbohydrate through gluconeogenesis, to the amino acid alanine and to ethanol. Unusual pyruvate levels can be found in various metabolic disorders and also liver diseases.

Method Principle
Pyruvate, by the action of Pyruvate Oxidase converts pyruvate in the presence of other components to acetyl phosphate with the release of hydrogen peroxide. The hydrogen peroxide thus produced is quantitatively determined by coupling 4-aminoantipyrine with N-ethyl-N-(2-hydroxy-3-sulphopropyl)-m-toluidine (TOOS) (3-4), where a quinonemine dye with maximum absorption at between 540-550 nm is produced (2). The following reaction scheme illustrates the reactions that occur in this method:

Pyruvate oxidase
Pyruvate +Pi +O_2 + H_2O ---------- Acetyl phosphate + CO_2 +H_2O_2

Peroxidase
$2H_2O_2$ + 4 APA + TOOS ----------→ Quinoneimine dye + $4H_2O$

PYRUVATE REAGENT
Each liter contains:

Buffer	
4-Aminoantipyrine (4AA)	0.15 mol
TOOS	1.2 mmol
Pyruvate Oxidase	≥ 200 Units
Peroxidase	≥ 5000 Units
Stabilizers, co-factors and nonreactive ingredients	

Precautions
Handle this reagent using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH**. Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage and Stability
Store the Catachem Pyruvate reagent at 2-8°C. When stored as directed the reagent is stable until expiration date stated on the label.

Working Reagent Preparation
The Catachem Pyruvate reagent is packaged in a powder format which is reconstituted with deionized/distilled water to form a single working reagent. Once opened and reconstituted, the Catachem Pyruvate Reagent is stable for at least 14 days at 2-8°C if stored capped.

Reagent Indications of Deterioration
- Turbidity
- Absorbance > 0.5 OD, 1 cm light path, 540/550nm
- Quality control values out of assigned ranges

If these reagent characteristics are observed contact Catachem technical service.

Specimen Collection and Stability (2, 3)
To maintain sample integrity and avoid changes in Pyruvate concentrations, care should be taken when collecting sample specimens.
Venous specimens should be collected without the use of a tourniquet or immediately after a tourniquet has been applied. Literature recommends sample collection in 6% perchloric acid in a chilled collection tube. (6)
Specimens should be collected, processed and analyzed promptly.

Procedure
These instructions are outlined for performing the Pyruvate assay by manual or automated procedures. Read the original instrument manufacturer's instructions and procedures before performing this as an automated pyruvate procedure.

Materials Provided
Catachem Pyruvate Reagent

Materials required but not Provided
- Analyzer (spectrophotometer) with 540 - 550nm wavelength
- Calibrator material with assigned pyruvate value
- Quality control material with assigned pyruvate values
- Deionized/distilled water

Calibration
Catachem Pyruvate Calibrator with assigned pyruvate value is recommended.

Calibration Schedule
Calibration should be performed when this method is run manually or implemented on an automated analyzer for the first time. Recalibration is required after changes of reagent lot number, major instrument service, and when quality control values are out of the indicated range.

Calibration Procedure
Instructions for calibrating any automated analyzer are provided by the specific instrument manufacturer. Read the entire recommended calibration procedure before proceeding with the instrument calibration.

Quality Control
To monitor the quality performance of the procedure, Catachem Pyruvate Control Level I and Level II with assigned pyruvate ranges should be included in the assay procedure each time the assay is performed.

Directions for Use
The Catachem Pyruvate method requires one reagent.

Procedure Limitations
Samples with Pyruvate values greater than 700 µmol/L should be diluted 1:2 with physiological saline and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

Interfering Substances
The following substances have no significant effect on the accuracy of this Pyruvate procedure at the concentrations stated.

• Hemoglobin	≤ 200 mg/dL
• Triglycerides	≤ 1000 mg/dL
• Bilirubin	≤ 2.2 mg/dL
• Lactate	≤ 200 µmol/L

353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone: (203) 262-0330 ● Fax: (203) 262-9836 ● email: catachem@catacheminc.com

Other substances and certain drugs may also influence the pyruvate values. (4)

Normal values (5)
In humans – 34-102 µmol/L (0.3-0.9 mg/dL)
In other animals, values vary depending on species

Analytical Parameters (Manual method)

Wavelength	540nm
Temperature	37˚ C
Path length	1 cm
Reaction Mode	End Point
Reaction Time	8 minutes
Reaction Volume	0.5 mL
Sample Volume	0.025 mL
Total Volume	0.525 mL
Sample-to-reagent ratio	1:20

Assay Procedure
1.With deionized/distilled water reconstitute the required volume of Pyruvate Working Reagent bottles needed dependent on the number of samples to be assayed.
2. Set spectrophotometer temperature at 37ºC, wavelength at 540nm.
3. Pipette 0.5 mL of Pyruvate reagent into each cuvette marked "Blank", "Test", "Calibrator" and "Control".
4. Pipette 0.025 mL of Blank (water), Test sample, Calibrator or Control into their respective cuvettes.
5. Mix all cuvettes and incubate all cuvettes for 5 minutes.
6. Read absorbance (A) of each cuvette marked "Blank", "Test", "Calibrator" and "Control" at the set wavelength of 540nm.

Pyruvate Procedure Scheme				
	RGT BLANK	**CAL**	**CONTROL**	**TEST**
Pyruvate Rgt.	**0.5 mL**	**0.5 mL**	**0.5 mL**	**0.5 mL**
Water (Blank)	**0.025 mL**	**-**	**-**	**-**
Sample	**-**	**0.025 mL**	**0.025 mL**	**0.025 mL**
Mix	**All cuvettes**			
Incubate	**All cuvettes at 37ºC for 5 minutes**			
Calculate	**Pyruvate µmol/L**			

Calculations and Results:

$$\text{Pyruvate (µmol/L)} = \frac{\text{(A) Sample - (A) Blank}}{\text{(A) Calibrator - (A) Blank}} \times \text{Calibrator (µmol/L)}$$

CATACHEM PYRUVATE REAGENT	
General BECKMAN AU TEST PARAMETERS	
Predilution Rate	**1**
Sample	**Serum**
S Volume	**7.5**
S Diluent	**0**
Repeat S Vol	*****
R1 Vol	**150**
R1 Diluent	**0**
R2 Vol	**-**
R2 Diluent	**0**
Wave Length Primary	**540 nm**
Wave Length Secondary	**700 nm**
Method Type/Reaction Slope	**END +**
Read Point Start 1	**0**
Read Point End 1	**27**
Read Point Start 2	**-**
Read Point End 2	**-**
Linearity Limit	*****
Reaction Min OD	**-2.0**
Reaction Max OD	**3.0**
Reagent OD Range 1-L	**-2**
Reagent OD Range 1-H	**2.5**
Reagent OD Range 2-L	**-2**
Reagent OD Range 2-H	**2.5**
Dynamic Range Low	**0**
Dynamic Range High	**700**
Normal L	*****
Normal H	*****
On Board Stability (Days #)	**14****
Cal Factor Low	**0**
Cal Factor High	**99999**
Calibration Type	**AB**
Formula	**Y=AX+B**
Calibration Replicates	
Cal 1	*****
Cal 2	
Cal Frequency Days	*****
Units	**µmol/L**

***User defined ** If Calibrated before each patient run**

Method Performance Characteristics

Linearity: This procedure is linear over the range of 0-700 µmol/L

Bibliography

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353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone: (203) 262-0330 ● Fax: (203) 262-9836 ● email: catachem@catacheminc.com