

ESERCITAZIONE:

Dosaggio del glucosio in siero

Mediante saggio spettrofotometrico

Parte 2

RICHIAMO DI TEORIA SULLA SPETTROFOTOMETRIA

Legge di Lambert-Beer:

$$A=abc$$

$A=$ **Assorbanza** = $\log(1/T)$. T =trasmissione= I/I_0 ADIMENSIONALE

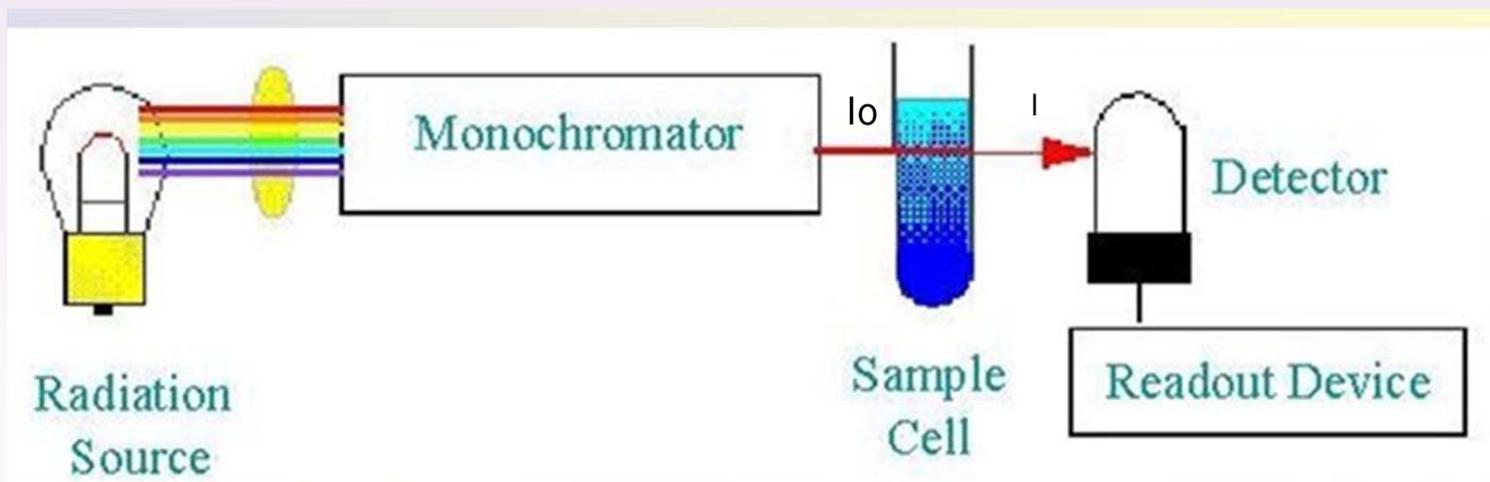
a =una data lunghezza d'onda, coefficiente di estinzione (molare oppure peso/volume) tipico della molecola [$M^{-1} \text{ cm}^{-1}$] oppure [$\text{ml mg}^{-1} \text{ cm}^{-1}$]

b = cammino ottico percorso dalla luce all'interno del campione [cm]

c = concentrazione del campione (molare oppure peso/volume) [M] oppure [mg ml^{-1}]

Dobbiamo quindi avere bene in mente se ricaveremo la concentrazione espressa in peso/volume o in molare.

Spettrofotometro tradizionale: 2 lampade
UV: 200-350 nm DEUTERIO
VIS 350-700 nm TUNGSTENO



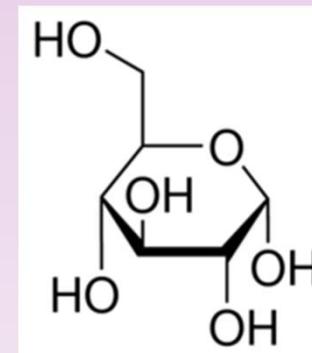
Quantificazione di glucosio in siero

In commercio sono disponibili diversi kit di saggio. Tutti i kit sono **enzimatici**. Gli enzimi utilizzati, che hanno come substrato il D-glucosio sono :

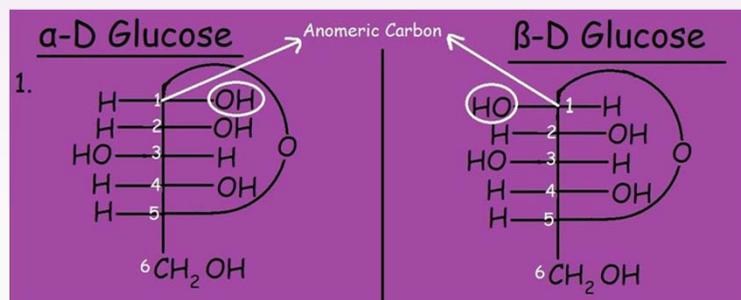
Glucosio ossidasi (GO)
Esochinasi (HK)



**METODI
COLORIMETRICI**

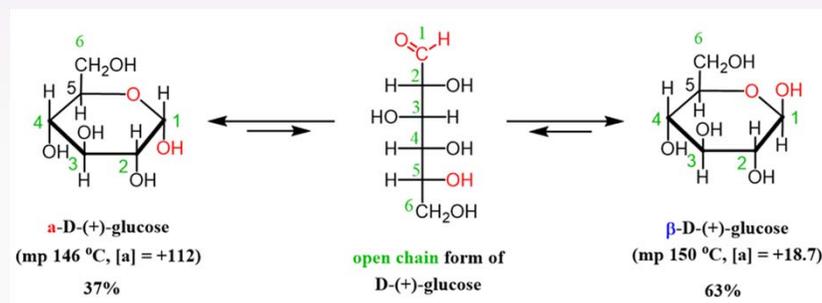


1) **Mutarotasi (catalizza l'interconversione tra anomeri alfa e beta)**



**METODO POLARIMETRICO
(attività ottica)**

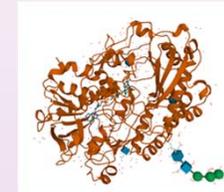
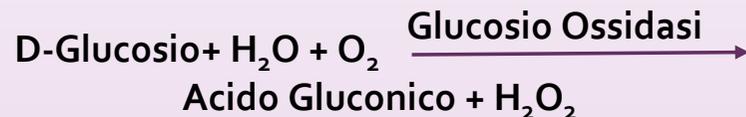
https://youtu.be/R4NHuE_ajNw?si=E_8kdMH0GIEBk6iv



METODO DELLA GLUCOSIO OSSIDASI

DOPPIA REAZIONE ENZIMATICA:

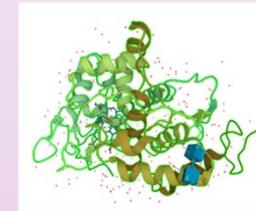
- 1) **GLUCOSIO OSSIDASI (GO)** catalizza l'ossidazione del glucosio in presenza di ossigeno con produzione di acido gluconico e perossido di idrogeno (H_2O_2)



- 1) la **PEROSSIDASI** catalizza l'ossidazione di o-dianisidina ridotta in presenza di H_2O_2 , con produzione di o-dianisidina ossidata (marrone, instabile)



o-dianisidina



- 1) l'acido solforico reagisce con la o-dianisidina ossidata formando un prodotto **di colore ROSA** stabile nel tempo



o-

O-dianisidina ossidata assorbe la luce a 540 nm (PIENA REGIONE VISIBILE)
=> l'**ASSORBANZA** a 540 nm è proporzionale alla concentrazione di glucosio presente

BIOSENSORI PER IL GLUCOSIO e CGM

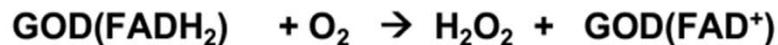
Continuous Glucose Monitoring systems

- La maggior parte dei biosensori per il glucosio sono basati sull'ossidazione del glucosio catalizzata dall'enzima glucosio-ossidasi (GOD).

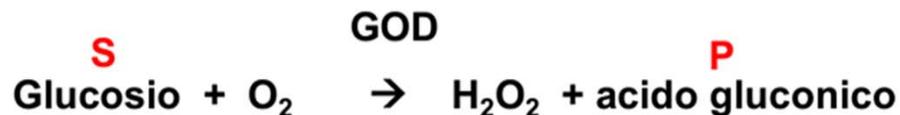
- L'enzima GOD, di solito estratto da funghi, ossida il glucosio secondo la reazione seguente



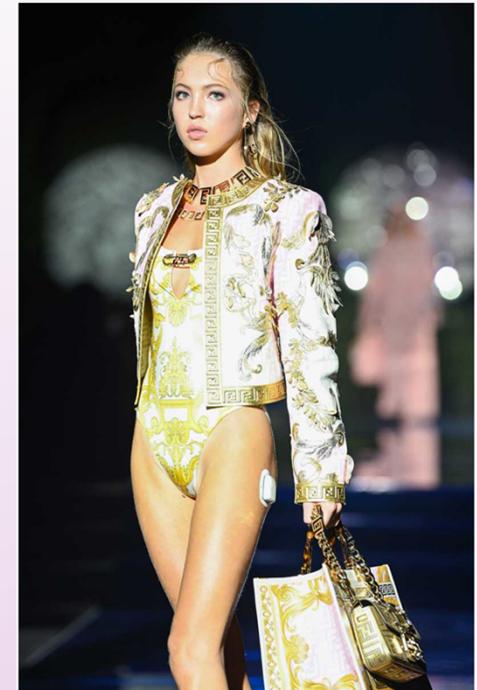
- Dove FAD è una flavina che funziona da cofattore dell'enzima GOD a cui è legato.
- Il GOD (FADH₂) è solitamente riossidato tramite reazione con ossigeno



- La sequenza di reazioni enzimatiche può essere riassunta come:



La concentrazione del prodotto può essere determinata con diversi metodi, una dei più utilizzati è quello elettrochimico in cui viene misurato la diminuzione di pH dovuta al prodotto (acido gluconico). Un'altra possibilità è quella di rilevare una riduzione locale della pressione parziale di O₂



Lila Moss in passerella per Versace con il cerotto CGM, 2021

Continuous Glucose Monitoring in Veterinary Patients

CGMs for companion animals with diabetes have become more commonplace in veterinary medicine as the advancement and affordability have progressed past more traditional methods.

<https://todaysveterinarypractice.com/diagnostics/continuous-glucose-monitoring-in-veterinary-patients/>



Figure 1. The adhesive side of a CGM sensor, after removal from a patient, showing the flexible polyurethane probe that stays in the interstitial space.

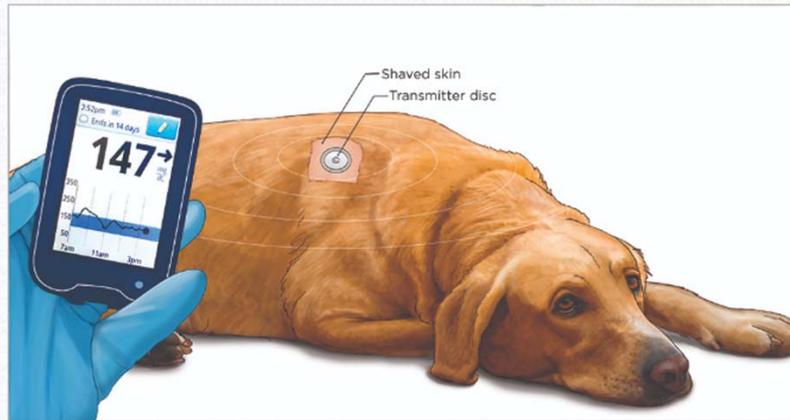


Figure 2. Components of a continuous glucose monitor. (B) The sensor records, stores, and transmits the data to the monitor. Illustration: Kip Carter

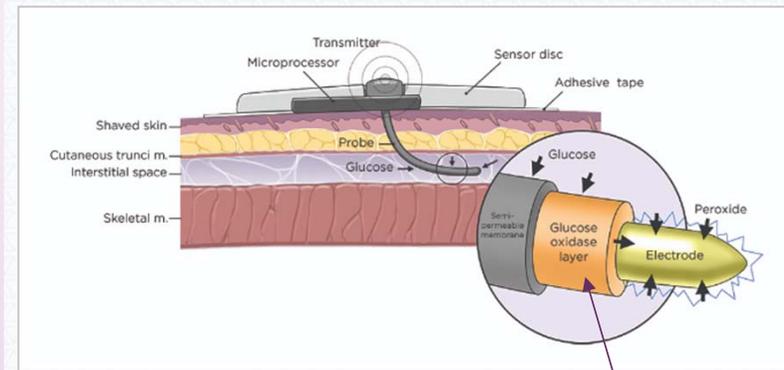


Figure 2. Components of a continuous glucose monitor. (A) The semipermeable membrane covering the subcutaneous probe allows glucose to pass through and come in contact with the inner layer, which contains glucose oxidase. The reaction of glucose with glucose oxidase creates hydrogen peroxide, generating an electrical current in direct proportion to the glucose concentration. The electrode at the center of the probe sends the signal to the external sensor, which translates the signal into a sensor glucose reading. Illustration: Kip Carter

Monitoring the feline diabetic with a continuous glucose monitor

As feline blood glucose is influenced by various factors such as stress in the veterinary clinic, a CGM can be useful to collect data over a longer period of time, including when stress-free at home



by Samantha Taylor
03 March 2022

 Read time: Approx 10 mins

<https://www.veterinary-practice.com/article/continuous-glucose-monitoring-feline-diabetes>

**GLUCOSE
OXIDASE**

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Open Access Case Report

Clinical Use of a 180-Day Implantable Glucose Monitoring System in Dogs with Diabetes Mellitus: A Case Series

by Antonio Maria Tardo ^{1,*}, Concetta Irace ², Francesca Del Baldo ¹, Armando Foglia ¹ and Federico Fracassi ¹

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² Department of Health Science, University Magna Graecia, 88100 Catanzaro, Italy

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Animals **2022**, *12*(7), 860; <https://doi.org/10.3390/ani12070860>

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(This article belongs to the Special Issue Animal Endocrinology and Medicine Research)



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AI nel trattamento del diabete

Computer Methods and Programs in Biomedicine Update
Volume 5, 2024, 100141

Artificial intelligence for diabetes: Enhancing prevention, diagnosis, and effective management

Mohamed Khalifa^{a,b,c}, Mona Albadawy^d

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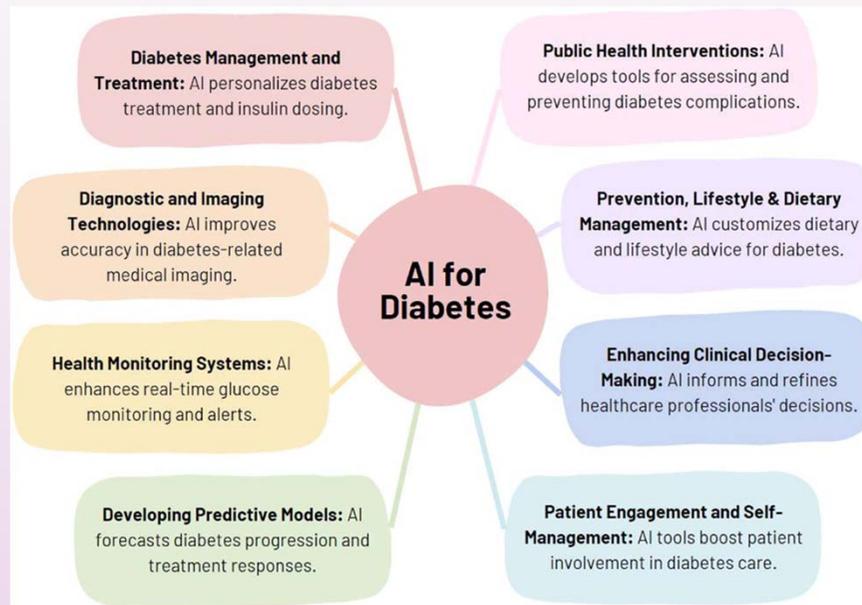
<https://doi.org/10.1016/j.cmpbup.2024.100141> Get rights and content

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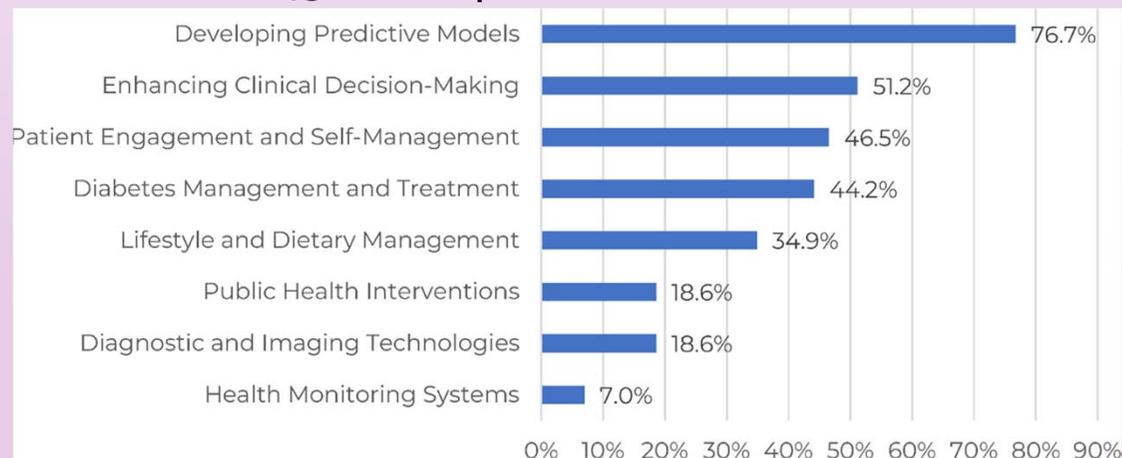
Highlights

- Artificial intelligence is revolutionizing diabetes care by predicting risks and customizing treatment plans to enhance patient outcomes.
- AI significantly impacts various aspects of diabetes management, including advanced diagnostics, predictive modeling, and personalized patient care.
- AI not only improves lifestyle and dietary management but also enhances clinical decisions and patient engagement for better diabetes management.
- The integration of AI in diabetes care marks a transformative shift towards a data-driven approach, promising improved patient care and outcomes.
- AI's role in diabetes care demands robust research, secure data practices, interdisciplinary teamwork, and strict ethical standards to optimize patient outcomes and ensure responsible implementation.

8 domini di interesse:



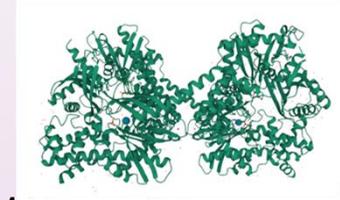
Risultati di 43 studi (pubmed):



METODO DELLA ESOCINASI

DOPPIA REAZIONE ENZIMATICA:

1) **ESOCINASI** catalizza la fosforilazione di glucosio dall' ATP con produzione di ADP e glucosio 6 fosfato (G6P)



1) Glucosio 6 fosfato deidrogenasi (**G6PDH**) catalizza l'ossidazione di G6P in presenza di NAD ossidata con produzione di 6-fosfogluconato e NADH ridotta.

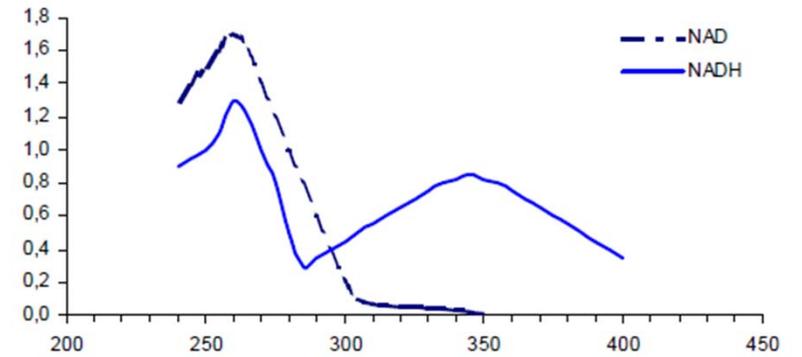
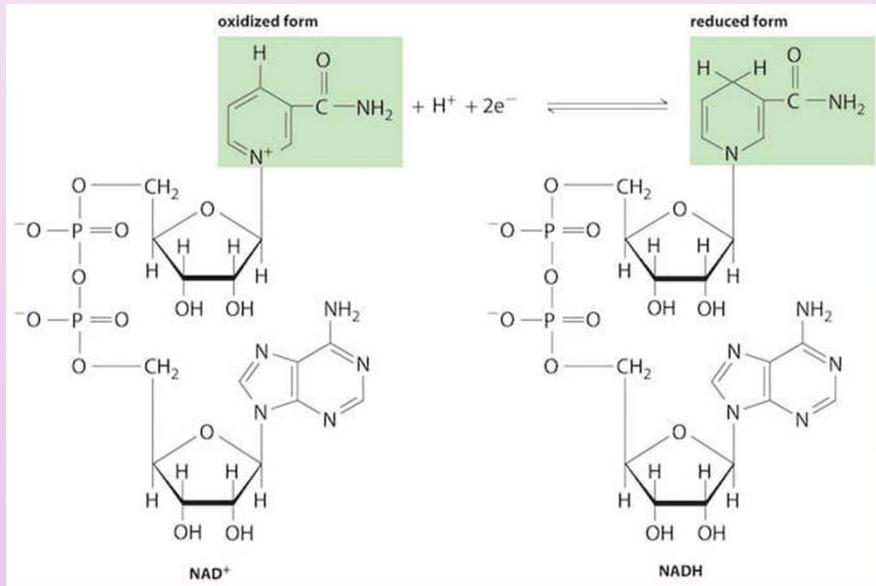
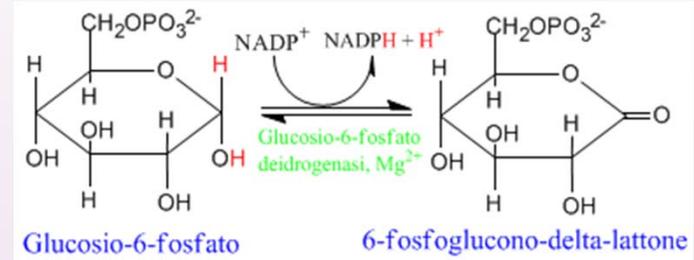
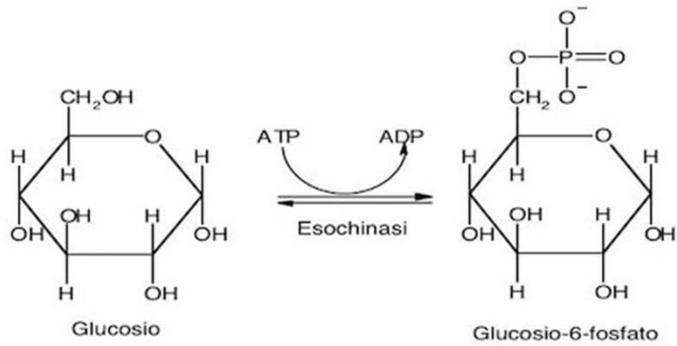


NADH assorbe luce a 340 nm

=> L'**ASSORBANZA** A 340 nm DEL PRODOTTO DELLA II reazione (NADH) è proporzionale alla concentrazione di glucosio presente in soluzione

=> SAGGIO ENZIMATICO COLORIMETRICO

NB 340 nm: Limite tra la regione UV e visibile. Regione dove le 2 lampade (UV e vis) di uno spettrofotometro tradizionale devono sovrapporre il segnale



<https://www.ncbi.nlm.nih.gov/books/NBK587446/>

3O8M

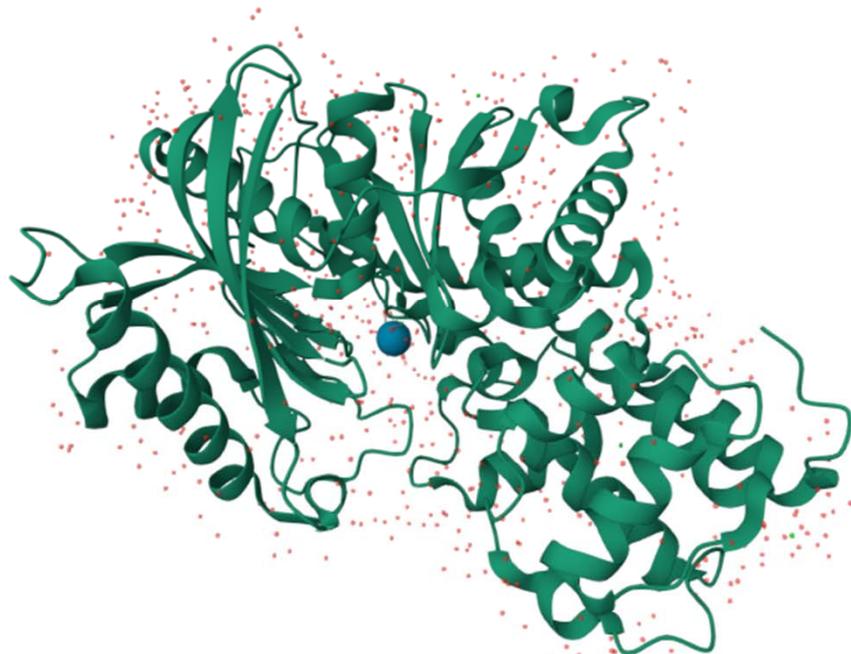
Crystal structure of monomeric KIHxk1 in crystal form XI with glucose bound (closed state)

Display Files Download Files

Help

Sequence of 3O8M | Crysta... Chain 1: Hexokinase A

```
MVRLGPKKPPARKGSMADVPANLMEQIHGLETLFTVSSEKMRISIVKHFISELDKGLSKKGGNIPMPGWWVEYPTGKETGDFLALDLGGINLRVVLKLGNGHDFDTTQNKY
RLPDHLRTGTSEQLWSFIAKCLKEFVDEWYDPGVSEPLPLGFTFSYPASQKKINSGLQRWTKGFDIEGVEGHVVVPMLEQIEKLNIPINVVVALINDTTGTLVASLYTDPQ
TKMGIIIGTVNGAYYDVVSGIEKLEGLLPEDIGPDSFMAINCEYGSFDNEHLVLPRTKYDVII DEESPRPQQQAFKMTSGYVLGEIMRLVLLDLVDYSGFIFKQDQISKLK
```



Structure

3O8M | Crystal structure of monome...

Type	Assembly
Asm Id	1: Author And Software ...
Dynamic Bonds	Off

Nothing Focused

Measurements

Structure Motif Search

Components

Preset	Add	Visibility	Actions
Polymer	Cartoon	Visible	...
Carbohydrate	2 reprs	Visible	...
Water	Ball & Stick	Visible	...
Ion	Ball & Stick	Visible	...

Unit Cell P 21 21 21

Density

Quality Assessment

Assembly Symmetry

Export Models

Export Animation

Export Geometry

https://www.rcsb.org/3d-view/5AQ1/1

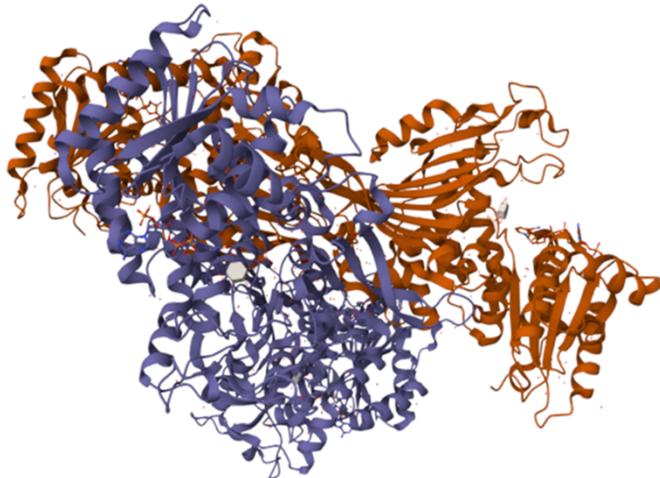
Display Files Download Files

Trypanosoma cruzi Glucose-6-phosphate Dehydrogenase in complex with G6P and NADPH

Help

Sequence of 5AQ1 | Trypan... Chain 1: GLUCOSE-6... B ASM_1

```
MGHHHHHHENLYFQGHMASGSDAVSPELRSFALTIIVVLGASGDLAKKGFPAFLFQLYCNGMLPRDWNILGYARSTMEDVERKWKDITLAGFFTRALDERGCHVGNFLRRI SYMT  
GSYDRDED FARLNERILRMEAFQGGPEKGNRLFYLALPFSVIVGVCRGLSKGAMQKPELGGWVRLIVEKPFGRDITETSEQLSNQLKPLFNERQVFRIDHYLGKEMVQNIIVT  
RFANRVFSALWNSNSIACVQIITFKEKIGTAGRGYFDSIGIIRDVIONHLTQILSLTMEKFRSLSAEDIRDEKVVQLRQVVPANFAECVVGQYASADGSTPGVLDLDPVSP
```



Structure

5AQ1 | Trypanosoma cruzi Glucose-6...

Type	Assembly
Asm Id	1: Author And Software ...
Dynamic Bonds	X Off
Nothing Focused	

Measurements

Structure Motif Search

Components 5AQ1

Preset	Add	Visibility	Actions
Polymer	Cartoon	☑	☰
Ligand	Ball & Stick	☑	☰
Carbohydrate	2 reprs	☑	☰
Water	Ball & Stick	☑	☰
Unit Cell	1 41 2 2	☑	☰

Density

Quality Assessment

Assembly Symmetry

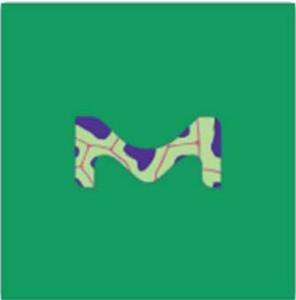
Export Models

Export Animation

Export Geometry

GAHK Assay KIT (Glucose Assay Hexokinase, Sigma-Aldrich Merck)

[← Indietro](#) Cannabis Testing Products For Cultivators, Producers & Testing Labs [GAHK20 >](#)



GAHK20  Supelco

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Glucose (HK) Assay Kit

★★★★★ (0) [Scrivi una recensione](#) [Fai una domanda](#)

sufficient for 20 assays

Autenticati per visualizzare i prezzi riservati alla tua organizzazione & contrattuali

Scegli un formato

Cambia visualizzazione  

1 KIT
98,90 €

NACRES: NA.84

Documenti

- [↓ SDS](#)
- [🔍 CdO/CdA](#)
- [Altri documenti >>](#)

Tutte le immagini (1)

Product Information

Glucose (HK) Assay Kit
 sufficient for 20 assays
 Catalog Number **GAHK20**

Glucose (HK) Assay Reagent
 Catalog Number **G3293**
 Storage Temperature 2–8 °C

TECHNICAL BULLETIN

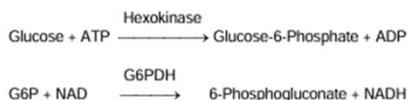
Product Description

Enzymes, as analytical tools, have found widespread use in the food, biochemical, and pharmaceutical industry. Enzymatic methods are specific, reproducible, sensitive, rapid, and therefore, ideal for analytical purposes. Because of the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation.

The Glucose (HK) Assay Kit is for the quantitative, enzymatic determination of glucose in food and other material. This kit has been used in various systems in studies related to topics such as:

- Hepatic glucose and lipid homeostasis in HEK cells⁴
- Maternal obesity models⁵
- Enzymatic fuel cell conversion of cellulose⁶

Principle



Glucose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase. Glucose-6-phosphate (G6P) is then oxidized to 6-phospho-gluconate in the presence of oxidized nicotinamide adenine dinucleotide (NAD), in a reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD is reduced to NADH. The consequent increase in absorbance at 340 nm is directly proportional to glucose concentration.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

1. Glucose (HK) Assay Reagent (Catalog Number G3293)
 - Reconstitute the vial contents with 20 mL of water.
 - After addition of water, stopper the vial and immediately mix several times by inversion.
 - DO NOT SHAKE.
 - When reconstituted with 20 mL of water, each vial contains 1.5 mM NAD, 1.0 mM ATP, 1.0 unit/mL of hexokinase, and 1.0 unit/mL of glucose-6-phosphate dehydrogenase, with sodium benzoate and potassium sorbate as preservatives.

The dry reagent is stored at 2–8 °C. The reagent should be discarded if:

- The vial contents exhibit caking, from possible moisture penetration
- The vial contents do not dissolve completely upon reconstitution
- Or if the reconstituted solution appears turbid

The reconstituted reagent is stable, in the absence of visible microbial growth, for 7 days at 18–26 °C and for at least 4 weeks at 2–8 °C. The reagent is not suitable for use if the absorbance of the freshly reconstituted solution measured at 340 nm vs. water as the reference is >0.350.

2. Glucose Standard Solution (Catalog Number G3285)
 - D-Glucose, 1.0 mg/mL in 0.1% benzoic acid.
 - This standard is traceable to an NIST standard and is supplied ready-to-use.
 - It is stable at 2–8 °C for at least six months. Discard if turbidity develops.

Equipment Required but Not Provided

1. Spectrophotometer suitable for measuring absorbance at 340 nm.
2. Cuvettes
3. Pipettes capable of accurately dispensing 10 µL to 1 mL.

Procedure

Sample Preparation:

Liquids:

- Dilute sample with deionized water to 0.05-5 mg of glucose/mL.
- Filter or deproteinize solution, if necessary, to clarify.
- Solutions that are strongly colored and that have a low glucose concentration should be decolorized.
- Carbonated or fermented products must be degassed.

Solids:

- Weigh out sample to nearest 0.1 mg.
- Extract sample with deionized water. The solution may be heated (<75 °C) to aid extraction.
- Dilute with deionized water to 0.05-5 mg of glucose/mL.
- Filter or deproteinize solution, if necessary, to clarify.

Determination:

- Pipette a volume of solution corresponding to 0.5-50 µg of glucose.
- Repeat the assay and vary the sample volume, if necessary, to give an ΔA_{340} between 0.03 and 1.6.
- Pipette the following solutions into the appropriately marked test tubes.

Tube	Glucose Assay Reagent (mL)	Sample Volume (µL)	Volume of Deionized Water (mL)
Sample Blank	–	Same as for Test	1.0
Reagent Blank	1.0	–	Same as Sample Volume for Test
Test	1.0	10–200	–

- Mix tubes and incubate for 15 minutes at room temperature (18–35 °C).
- Measure the absorbance at 340 nm versus deionized water.

Calculations:

The total blank must take into account the contribution to the absorbance of the sample and the Glucose Assay Reagent.

$$A_{\text{Total Blank}} = A_{\text{Sample Blank}} + A_{\text{Reagent Blank}}$$

$$\text{mg glucose/mL} = \frac{(\Delta A) (TV) (\text{Glucose Molecular Weight}) (F)}{(\epsilon)(d)(SV) (\text{Conversion Factor for } \mu\text{g to mg})}$$

$$\text{mg glucose/mL} = \frac{(\Delta A) (TV) (180.2) (F)}{(6.22) (1) (SV) (1,000)}$$

$$\text{mg glucose/mL} = \frac{(\Delta A) (TV) (F) (0.029)}{(SV)}$$

$$\Delta A = A_{\text{Test}} - A_{\text{Total Blank}}$$

$$TV = \text{Total Assay Volume (mL)}$$

$$SV = \text{Sample Volume (mL)}$$

$$\text{Glucose MW} = 180.2 \text{ g/mole or equivalently } 180.2 \mu\text{g}/\mu\text{moles}$$

$$F = \text{Dilution Factor from Sample Preparation}$$

$$\epsilon = \text{Millimolar Extinction Coefficient for NADH at 340 nm Millimolar}^{-1} \text{ cm}^{-1} \text{ or equivalently (mL}/\mu\text{moles})(1/\text{cm})$$

$$d = \text{Light path (cm)} = 1 \text{ cm}$$

$$1,000 = \text{Conversion Factor for } \mu\text{g to mg}$$

References

1. Bondar, R.J.L., and Mead, D.C., *Clin. Chem.*, **20(5)**, 586-590 (1974).
2. Kunsst, A. et al., *Methods of Enzymatic Analysis*, 3rd Edition (H.U. Bergmeyer, ed.). Academic Press (New York, NY), Vol. 2, pp. 163-172 (1984).
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5. Chen, J.-R. et al., *Endocrinology*, **157(11)**, 4172-4183 (2016).
6. Chen, Q. et al., *J. Biotech.*, **263**, 30-35 (2017).

COMPONENTI:

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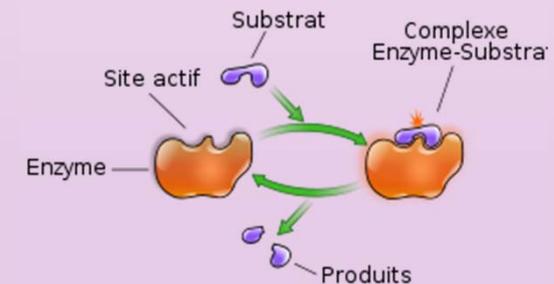
1. REAGENTE DI SAGGIO (ricostituito con 20 ml di H₂O)

Ogni vial contiene:

- 1.5 mM NAD
- 1.0mM ATP
- 1.0 unità/mL di enzima hexokinase (HK)*
- 1.0 unità/mL di enzima glucosio-6- fosfato deidrogenasi (G6DPH)*

2. Soluzione di glucosio da utilizzare come standard:
D-glucosio alla concentrazione di 1 mg/ml in acido benzoico 0.1%

* ricordiamo che 1 unità enzimatica è definita come la quantità di un enzima che catalizza la conversione di 1 micro mole di substrato in un minuto alla temperatura di 25 °C



Equipment Required but Not Provided

1. Spectrophotometer suitable for measuring absorbance at 340 nm.
2. Cuvettes
3. Pipettes capable of accurately dispensing 10 μL to 1 mL.

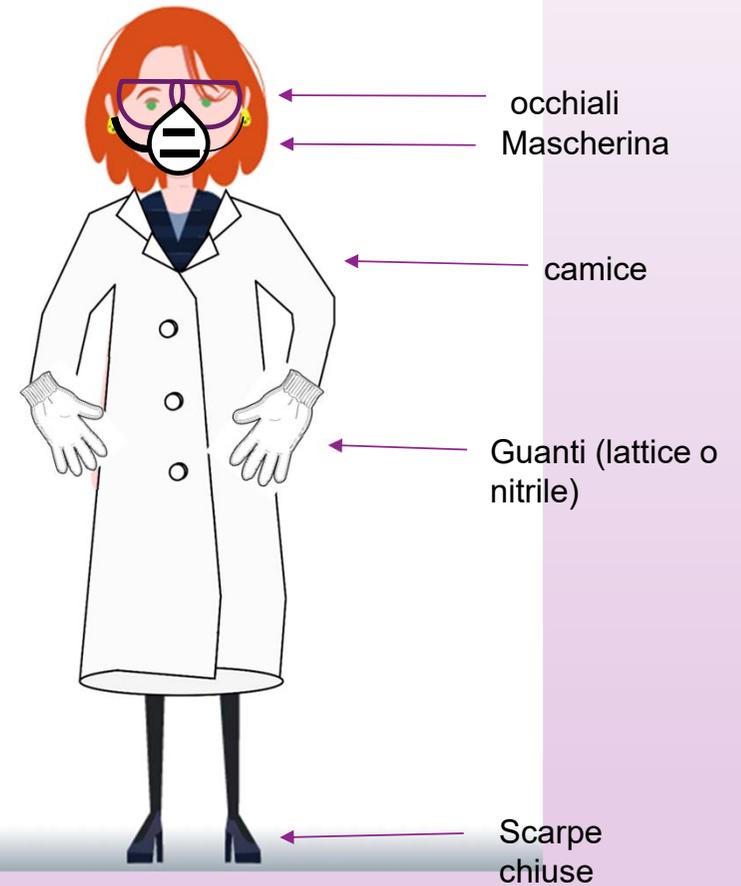
4. provette (eppendorf) da 1,5 ml o 2 ml

4. DISPOSITIVO ELETTRONICO (PC, tablet, smartphone) dotato di foglio di calcolo!!!!

0.

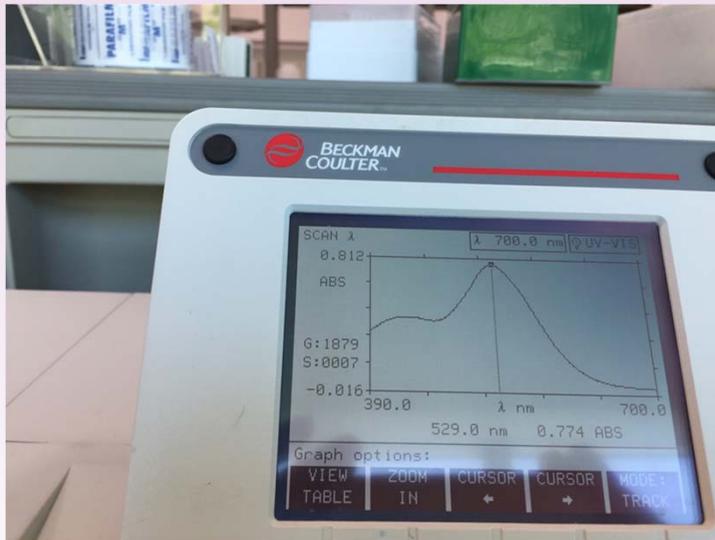
D

PI



1. SPETTROFOTOMETRO

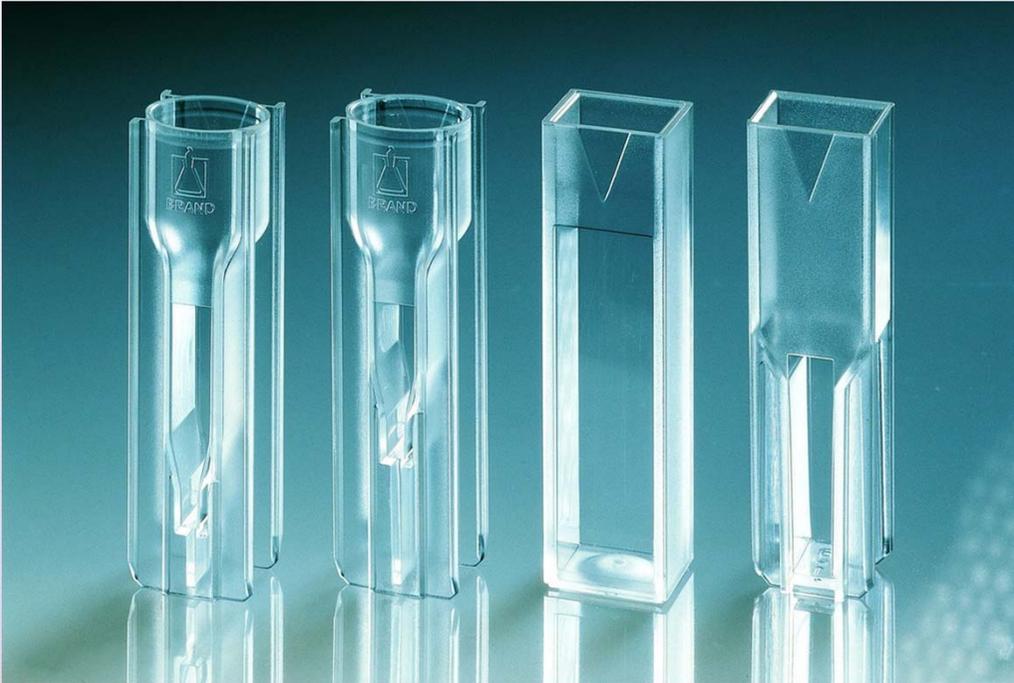
Observed Color of Compound	Color of Light Absorbed	Approximate Wavelength of Light Absorbed
Green	Red	700 nm
Blue-green	Orange-red	600 nm
Violet	Yellow	550 nm
Red-violet	Yellow-green	530 nm
Red	Blue-green	500 nm
Orange	Blue	450 nm
Yellow	Violet	400 nm



CUVETTA (singolo canale)

PIASTRA

2. CUVETTE



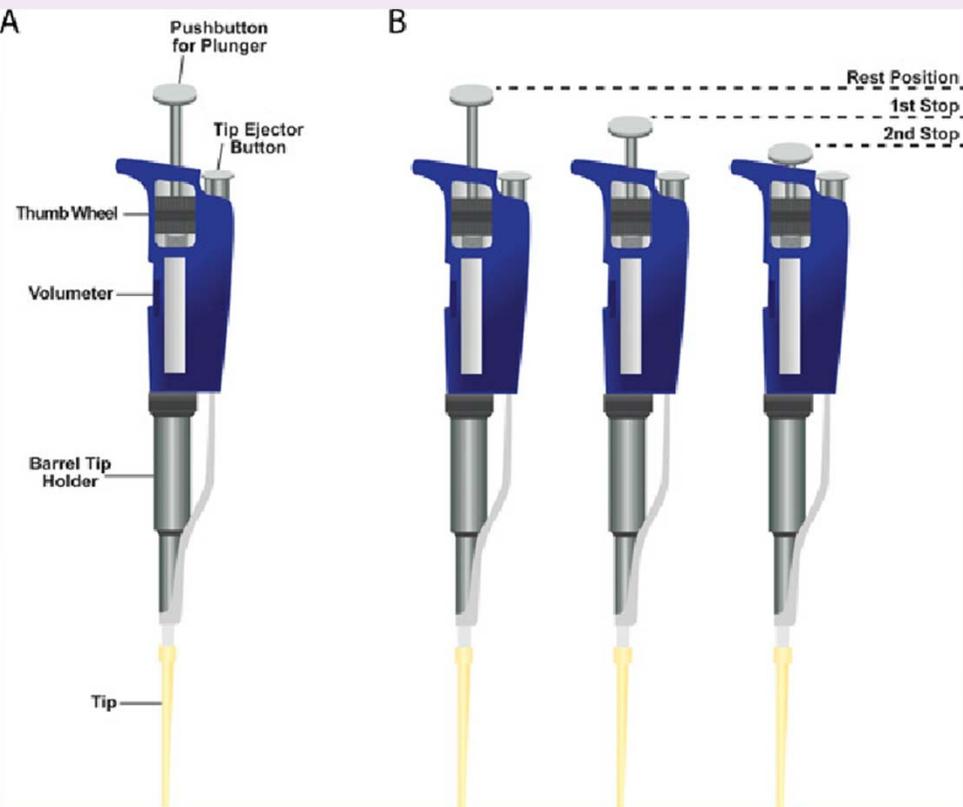
MATERIALE PLASTICO
TRASPARENTE ALLA LUCE VISIBILE.
USA E GETTA

Notare l' altezza della finestra e la
presenza di una freccetta.
Il cammino ottico deve essere di 1 cm,
quindi la direzione della luce è obbligata!

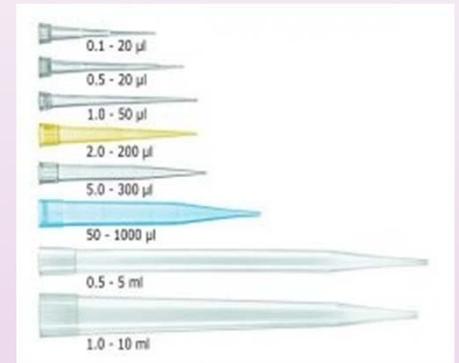
3. MICROPIPETTE

<https://youtu.be/3d-U9vowVwg>
PIPETTING SKILLS

Micropipetta



Puntali



4. PROVETTE DA CENTRIFUGA GRADUATE (Eppendorf)

Capacità 0,5 ml; 1,5 ml; 2,0 ml

RICORDA: 1 mL = 1 cm³



● HK PREPARAZIONE DEL CAMPIONE

Procedure

Sample Preparation:

Liquids:

- Dilute sample with deionized water to 0.05-5 mg of glucose/mL.
- Filter or deproteinize solution, if necessary, to clarify.
- Solutions that are strongly colored and that have a low glucose concentration should be decolorized.
- Carbonated or fermented products must be degassed.

Solids:

- Weigh out sample to nearest 0.1 mg.
- Extract sample with deionized water. The solution may be heated (<75 °C) to aid extraction.
- Dilute with deionized water to 0.05-5 mg of glucose/mL.
- Filter or deproteinize solution, if necessary, to clarify.

DILUIRE IL CAMPIONE
ad una concentrazione di glucosio
0.05-5 mg/ml

DOMANDE:

Il mio campione ha una concentrazione di glucosio compresa nell'intervallo dato?

Perchè devo avere concentrazioni di glucosio che rientrino in un determinato intervallo?

HK

1) Consultiamo i valori di riferimento della glicemia specie specifici

CANE 60-110 mg/dL= 0,6-1,1 mg/ml

GATTO 70-110 mg/dL=0,7-1,1 mg/ml

CAVALLO 77-132 mg/dL= 0,77-1,32 mg/ml

=> possiamo usare un'aliquota di campione tal quale

2) DEVO AVERE VALORI DI CONCENTRAZIONE COMPRESI IN UN DETERMINATO RANGE per avere valori di assorbanza nel range di capacità di lettura dello strumento

Test	Unità	Specie	Valori di riferimento	
Esame microscopico del sedimento urinario	Nitriti			
	Entrociti		$10^6/\mu\text{L}$	
	Leucociti		$10^3/\mu\text{L}$	
	Cellule epiteliali			
	Cilindri			
Ferro (Sideremia)	microg/dL	cane gatto cavallo	100 - 220 68 - 215 74 - 209	
Fosfatasi alcalina	U/L	cane gatto cavallo	≤ 280 25 - 93 ≤ 285	
Fosforo	mg/dL	cane gatto cavallo	2.5 - 4.7 4.0 - 5.2 2.0 - 4.3	
Fruttosammina	micromoli/L	cane	non diabetico diabetico	225 - 365 320 - 850
		gatto	non diabetico diabetico	190 - 365 350 - 740
Gamma-glutammi-transferasi (GGT)	U/L	cane gatto cavallo	≤ 12 ≤ 5 ≤ 23	
Glicemia	mg/dL	cane gatto cavallo	60 - 110 70 - 110 77 - 132	
Potassio	mEq/L	cane gatto cavallo	4.0 - 5.2 3.0 - 4.8 2.8 - 4.5	
Lattico Deidrogenasi (LDH)	U/L	cane gatto cavallo	≤ 100 ≤ 100 ≤ 380	
Lipasi	U/L	cane gatto cavallo	≤ 250	
Magnesio	mg/dL	cane	1.0 - 2.2	
Proteine totali	g/dL	cane gatto cavallo	6.0 - 7.6 5.8 - 7.8 6.1 - 8.0	
Proteine urinarie	mg/dL	cane	5 - 80	
Proteine totali	g/dL	cane	5.4 - 7.6	
		gatto	5.4 - 7.8	
		cavallo	6.1 - 8.0	
Albumina	%	cane	50 - 60	
		gatto	45 - 55	
		cavallo	40 - 65	
Albumina	g/dL	cane	2.20 - 3.60	
		gatto	2.20 - 3.20	

PROTOCOLLO

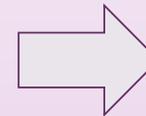
PROTOCOLLO del datasheet:
totale 20 saggi,
volume di saggio 1010-1200
microlitri per ogni campione

Determination:

- Pipette a volume of solution corresponding to 0.5-50 μg of glucose.
- Repeat the assay and vary the sample volume, if necessary, to give an ΔA_{340} between 0.03 and 1.6.
- Pipette the following solutions into the appropriately marked test tubes.

Tube	Glucose Assay Reagent (mL)	Sample Volume (μL)	Volume of Deionized Water (mL)
Sample Blank	–	Same as for Test	1.0
Reagent Blank	1.0	–	Same as Sample Volume for Test
Test	1.0	10–200	–

- Mix tubes and incubate for 15 minutes at room temperature (18–35 $^{\circ}\text{C}$).
- Measure the absorbance at 340 nm versus deionized water.



Adattiamo il
protocollo per un
volume totale di 200
microlitri per ogni
campione

CALCOLI

Calculations:

The total blank must take into account the contribution to the absorbance of the sample and the Glucose Assay Reagent.

$$A_{\text{Total Blank}} = A_{\text{Sample Blank}} + A_{\text{Reagent Blank}}$$

$$\text{mg glucose/mL} = \frac{(\Delta A) (TV) (\text{Glucose Molecular Weight}) (F)}{(\epsilon)(d)(SV)(\text{Conversion Factor for } \mu\text{g to mg})}$$

$$\text{mg glucose/mL} = \frac{(\Delta A) (TV) (180.2) (F)}{(6.22) (1) (SV) (1,000)}$$

$$\text{mg glucose/mL} = \frac{(\Delta A) (TV) (F) (0.029)}{(SV)}$$

$$\Delta A = A_{\text{Test}} - A_{\text{Total Blank}}$$

TV = Total Assay Volume (mL)

SV = Sample Volume (mL)

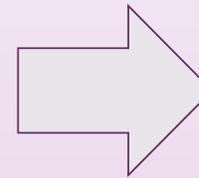
Glucose MW = 180.2 g/mole or equivalently
180.2 $\mu\text{g}/\mu\text{moles}$

F = Dilution Factor from Sample Preparation

ϵ = Millimolar Extinction Coefficient for NADH at 340 nm
Millimolar⁻¹ cm⁻¹ or equivalently (mL/ μmoles)(1/cm)

d = Light path (cm) = 1 cm

1,000 = Conversion Factor for μg to mg



Semplifichiamo questa formula e analizziamo i fattori partendo dalla legge di Lambert-Beer