# ESERCITAZIONE:
# Dosaggio del glucosio in siero
# Mediante saggio spettrofotometrico
# Parte 2

# RICHIAMO DI TEORIA SULLA SPETTROFOTOMETRIA

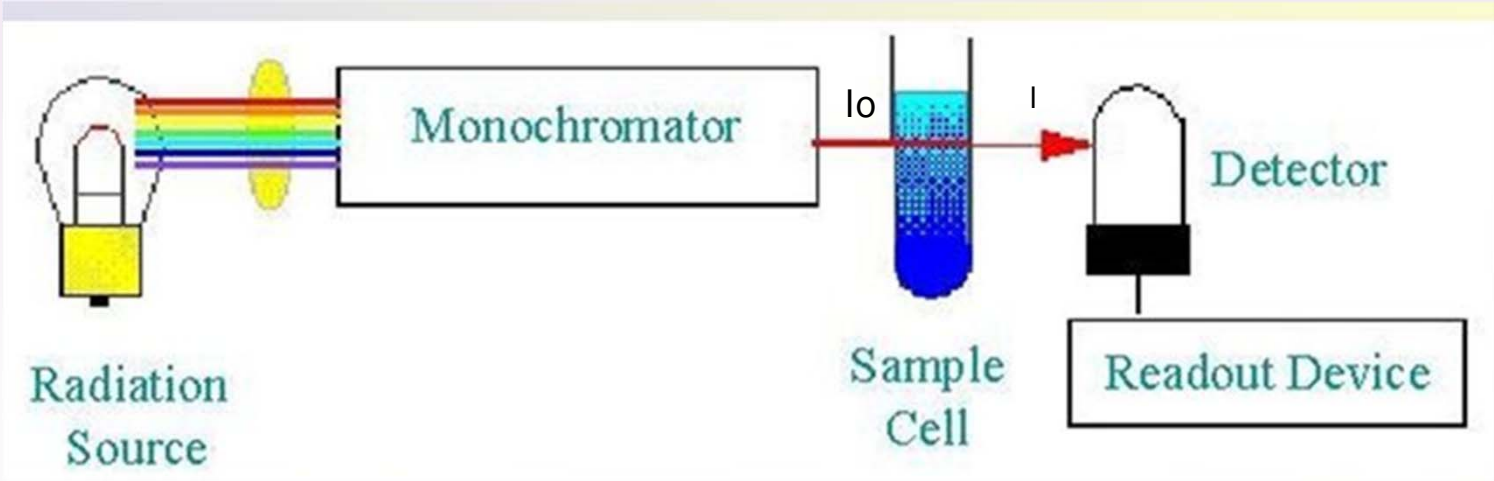
Legge di Lambert-Beer:

A=abc

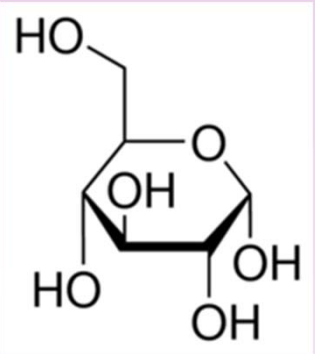
A= **Assorbanza** = log (1/T) . T=trasmittanza=$I/I_0$ ADIMENSIONALE
a=una data lunghezza d'onda, coefficiente di estinzione (molare oppure peso/volume) tipico della molecola [$M^{-1}$ $cm^{-1}$] oppure [ml $mg^{-1}$ $cm^{-1}$]
b= cammino ottico percorso dalla luce all'interno del campione [cm]
c= concentrazione 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

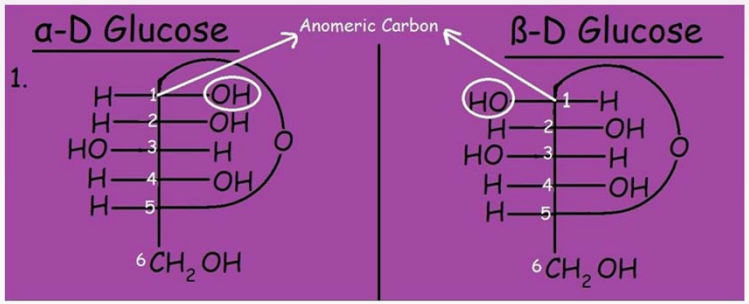



**In commercio sono disponibili diversi kit di saggio.**
**Tutti i kit sono enzimatici.**
**Gli enzimi utilizzati, che hanno come substrato il D-glucosio sono :**

**Glucosio ossidasi (GO)**
**Esochinasi (HK)**

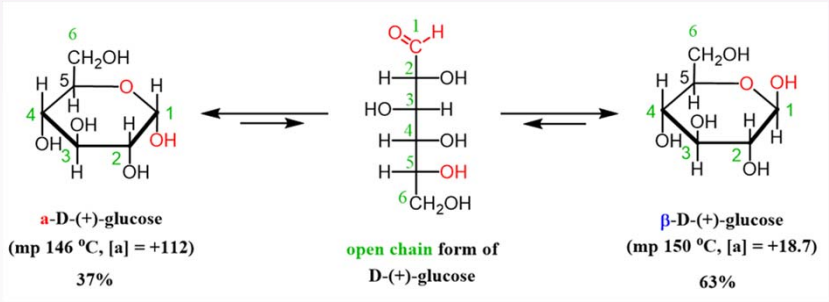
**METODI COLORIMETRICI**

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




**METODO POLARIMETRICO (attività ottica)**

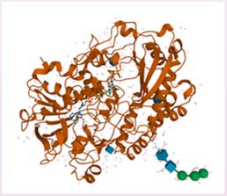
https://youtu.be/R4NHuE_ajNw?si=E_8kdMH0GIEBk6iv



# METODO DELLA GLUCOSIO OSSIDASI

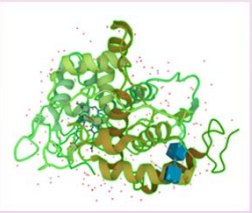
DOPPIA REAZIONE ENZIMATICA:

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



**D-Glucosio+ $H_2O$ + $O_2$** —(**Glucosio Ossidasi**)→ **Acido Gluconico + $H_2O_2$**

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



**$H_2O_2$+ o-dianisidina ridotta** —(**Perossidasi**)→ **o-dianisidina ossidata**

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

**o-dianisidina ossidata (marrone)** —($H_2SO_4$)→ **o-dianisidina (ROSA)**

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 catalizzato dall'enzima glucosio-ossidasi (GOD).
  - L'enzima GOD, di solito estratto da funghi, ossida il glucosio secondo la reazione seguente

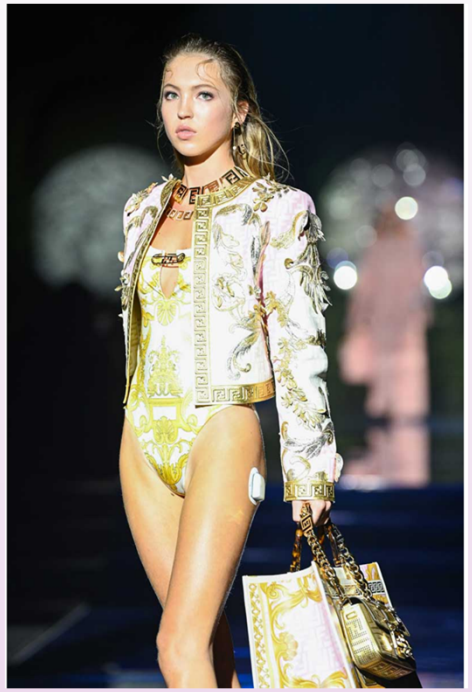
    **Glucosio + GOD($FAD^+$) → acido gluconico + GOD ($FADH_2$)**
  - Dove FAD è una flavina che funziona da cofattore dell'enzima GOD a cui è legato.
  - Il GOD (FADH2) è solitamente riossidato tramite reazione con ossigeno

    **GOD($FADH_2$) + $O_2$ → $H_2O_2$ + GOD($FAD^+$)**
- La sequenza di reazioni enzimatiche può essere riassunta come:

$$\text{Glucosio (S)} + O_2 \xrightarrow{\text{GOD}} H_2O_2 + \text{acido gluconico (P)}$$

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 $O_2$





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

TVP
TODAY'S VETERINARY PRACTICE

# 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.

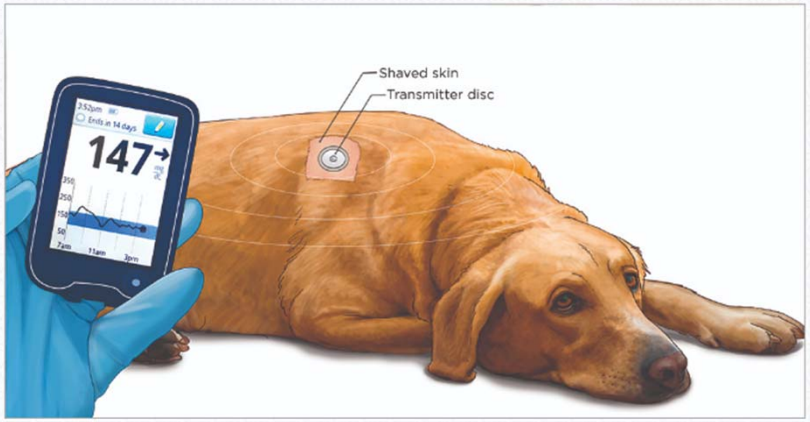



Figure 2. Components of a continuous glucose monitor. (B) The sensor records, stores, and transmits the data to the monitor. Illustration: Kip Carter

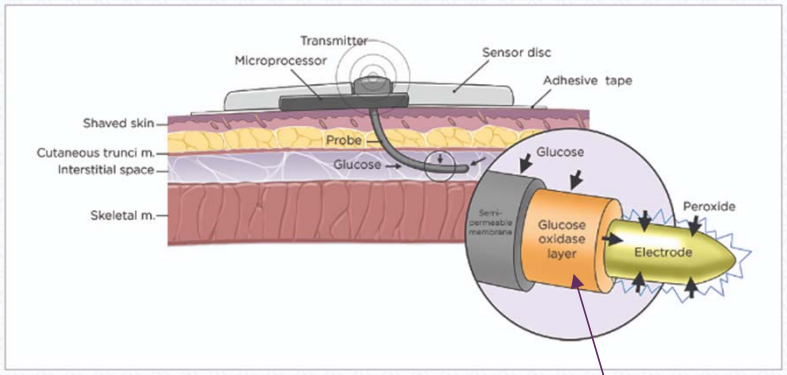



Figure 2. Components of a continuous glucose monitor. (A) The semipermeable membrane covering the subcutaneous probe allows glucose to pass through and come in contact with the inner layer, which contains glucose oxidase. The reaction of glucose with glucose oxidase creates hydrogen peroxide, generating 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

Improve Veterinary Practice

# 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

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]

[1] Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, 40064 Bologna, Italy

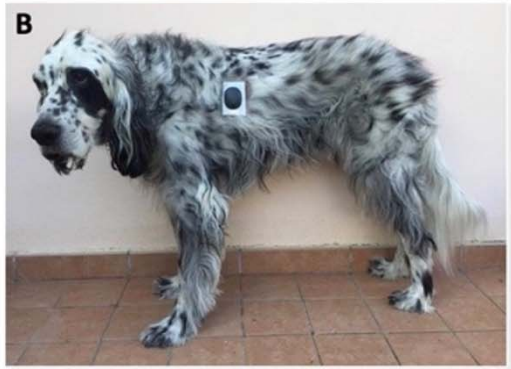
[2] Department of Health Science, University Magna Græcia, 88100 Catanzaro, Italy

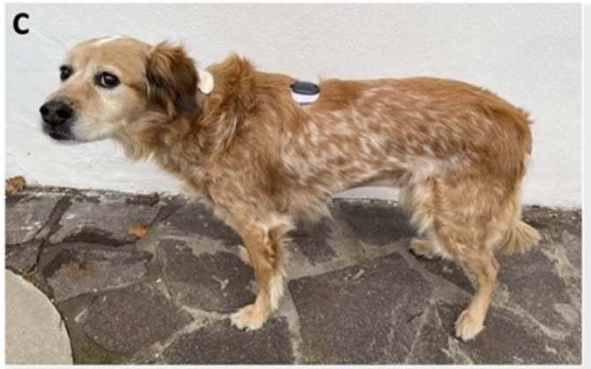
* Author to whom correspondence should be addressed.

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

**Received: 10 December 2021 / Revised: 24 March 2022 / Accepted: 28 March 2022 / Published: 29 March 2022**

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

# AI nel trattamento del diabete

ELSEVIER

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]

Show more

+ Add to Mendeley  Share  Cite

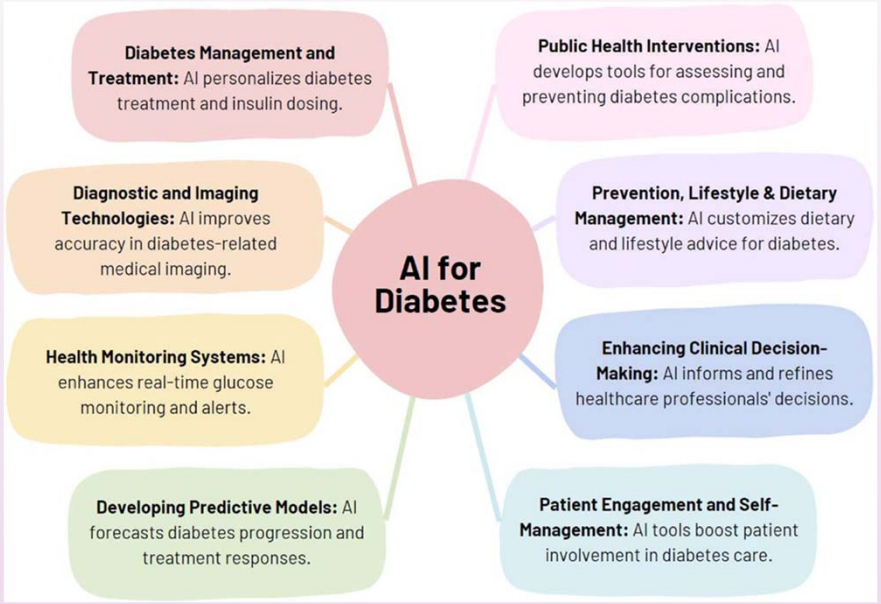
https://doi.org/10.1016/j.cmpbup.2024.100141  Get rights and content

Under a Creative Commons license  open access

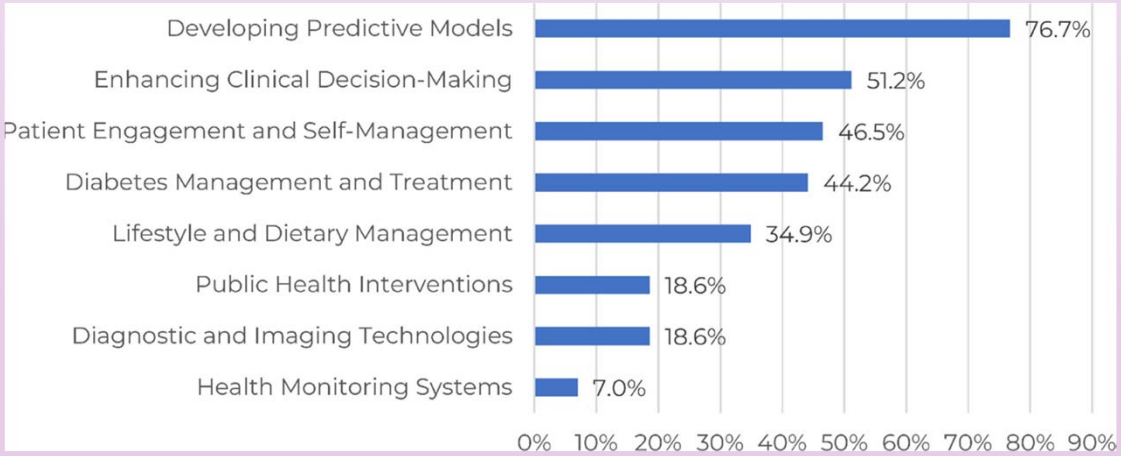
### Highlights

- 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):



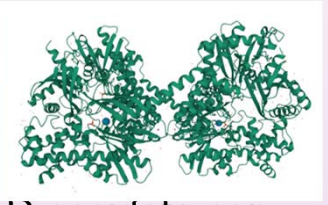
# METODO DELLA ESOCHINASI

DOPPIA REAZIONE ENZIMATICA:

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

**Esochinasi**

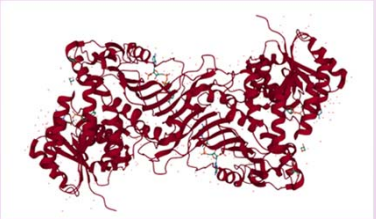
**Glucosio + ATP → Glucosio-6-Fosfato (G6P) + ADP**



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

**G6PDH**

**G6P+ NAD → 6-Fosfogluconato + NADH**

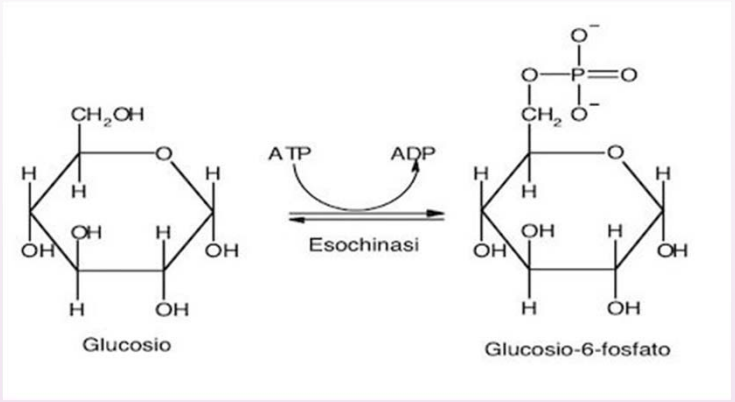


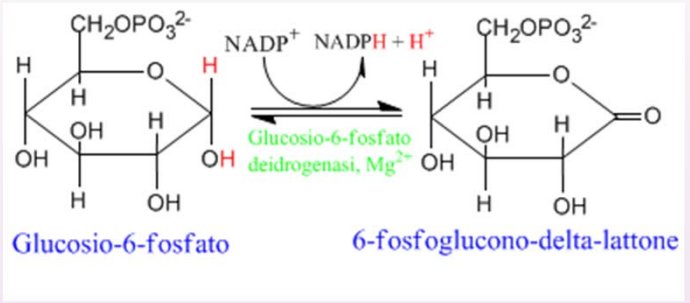
NADH assorbe luce a 340 nm

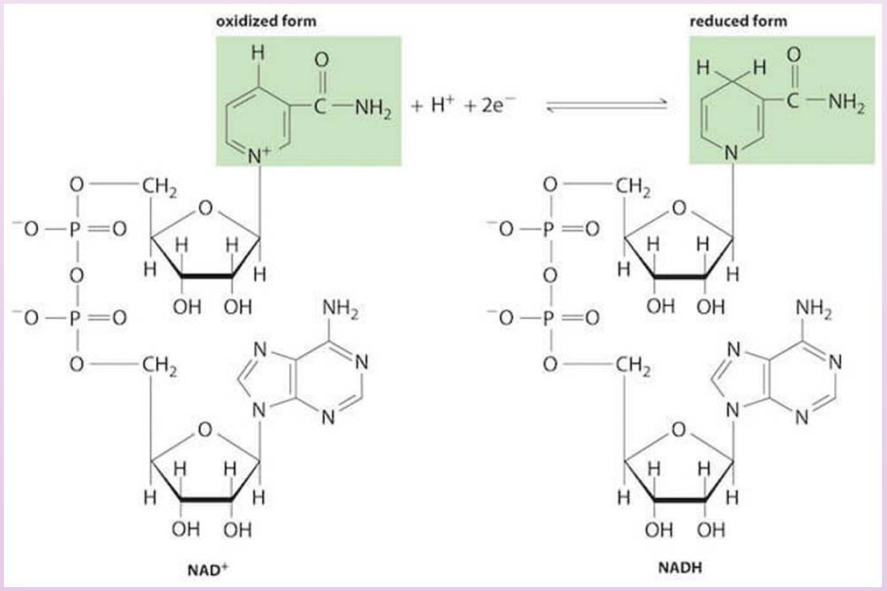
=> L'**ASSORBANZA** A 340 nm DEL PRODOTTO DELLA II reazione (NADH) è proporzionale alla concentrazione di glucosio presente in soluzione

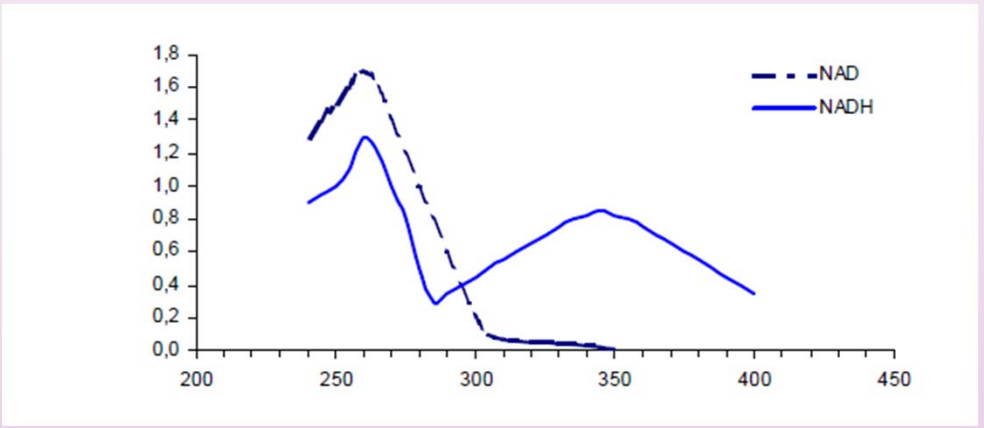
=> SAGGIO ENZIMATICO COLORIMETRICO

**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**



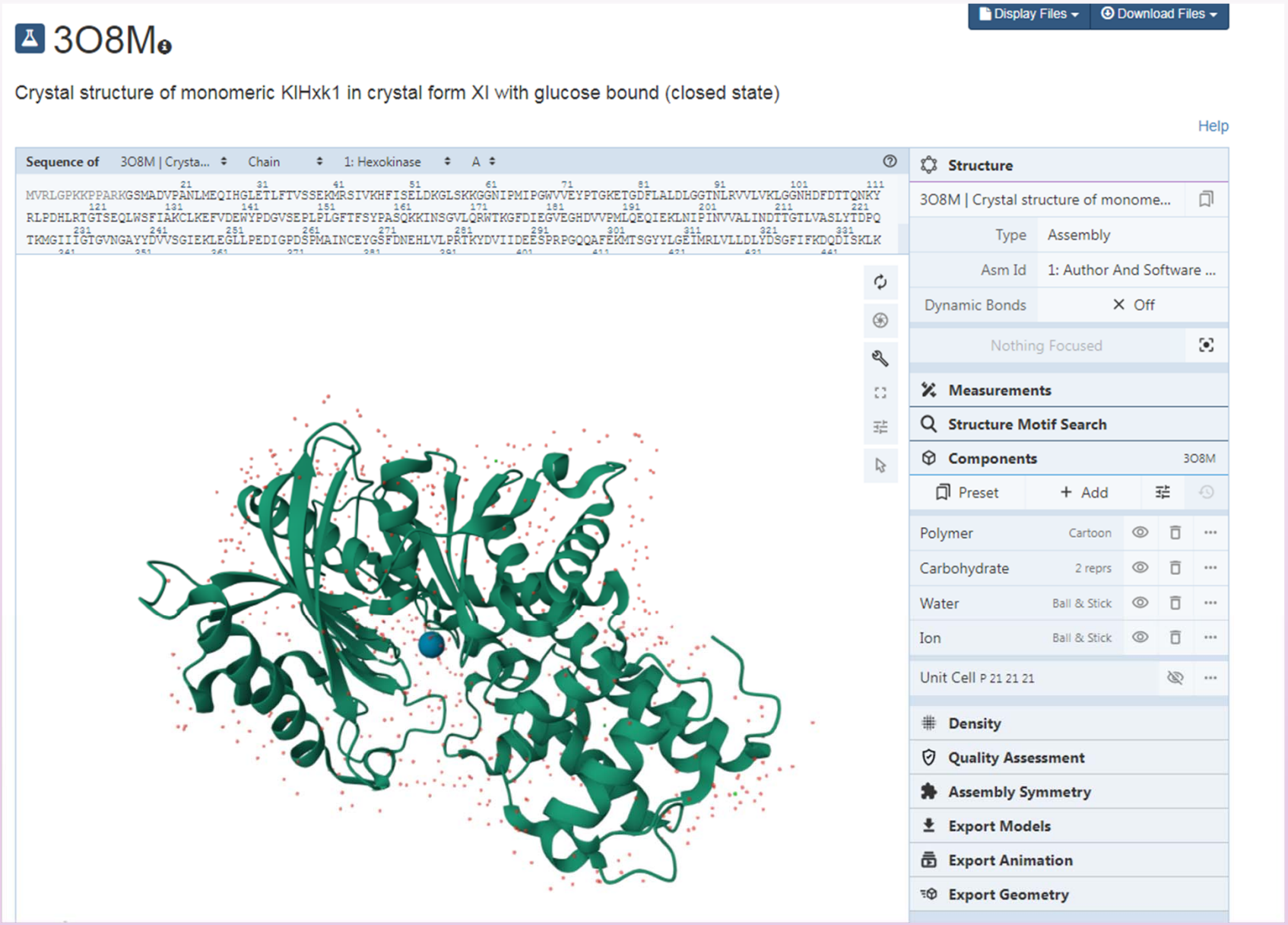



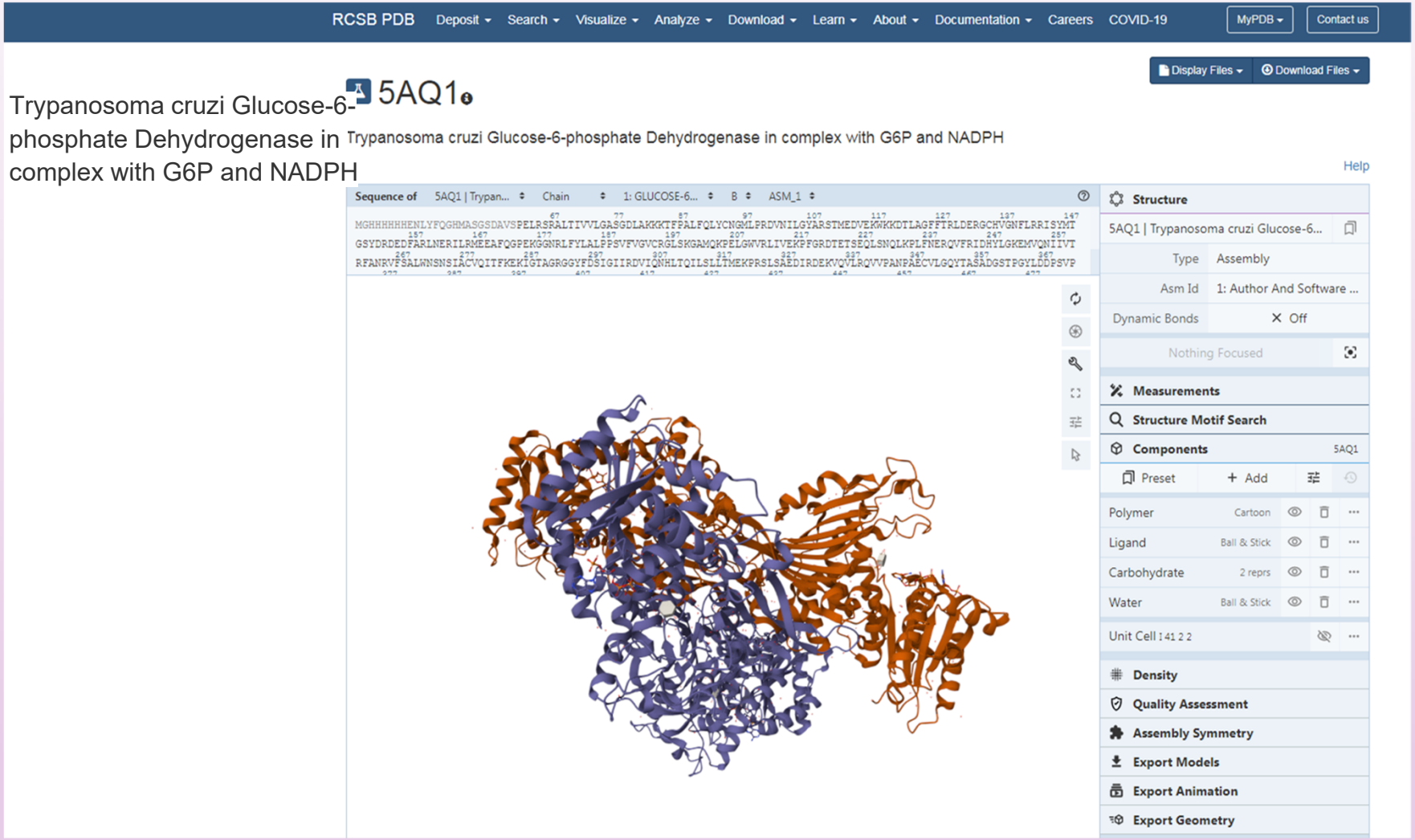

https://www.ncbi.nlm.nih.gov/books/NBK587446/

https://www.rcsb.org
/3d-view/3O8M/1



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

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



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

SIGMA-ALDRICH®
sigma-aldrich.com
3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# 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 cells[4]
- Maternal obesity models[5]
- Enzymatic fuel cell conversion of cellulose[6]

Principle

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-Phosphate} + \text{ADP}$$

$$\text{G6P} + \text{NAD} \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate} + \text{NADH}$$

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 ΔA340 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.

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

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

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

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

$\Delta A = A_{\text{Test}} - A_{\text{Total Blank}}$
TV = Total Assay Volume (mL)
SV = Sample Volume (mL)
Glucose MW = 180.2 g/mole or equivalently 180.2 µg/µmoles
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

### 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).
3. Southgate, D.A.T., *Determination of Food Carbohydrates*. Applied Science Publishers (London, UK: 1976).
4. Cheng, Y.-S. *et al.*, *PLoS Genet.*, **11(10)**, e1005561 (2015).
5. Chen, J.-R. *et al.*, *Endocrinology*, **157(11)**, 4172-4183 (2016).
6. Chen, Q. *et al.*, *J. Biotech.*, **263**, 30-35 (2017).

CMH,GCY,MAM 12/18-1

# COMPONENTI:

**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.

2. Glucose Standard Solution (Catalog Number G3285)

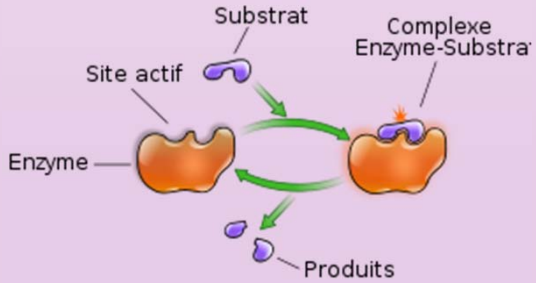
- D-Glucose, 1.0 mg/mL in 0.1% benzoic acid.
- This standard is traceable to an NIST standard and is supplied ready-to-use.
- It is stable at 2–8 °C for at least six months. Discard if turbidity develops.

1. REAGENTE DI SAGGIO (ricostituito con 20 ml di $H_2O$)

   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.

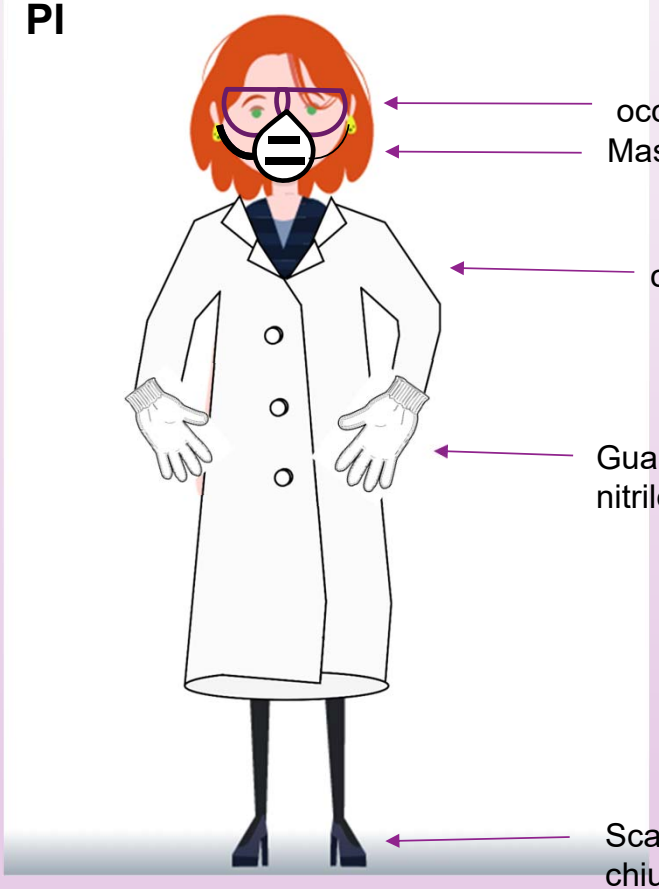
4. provette (eppendorf) da 1,5 ml o 2 ml

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

**0.**

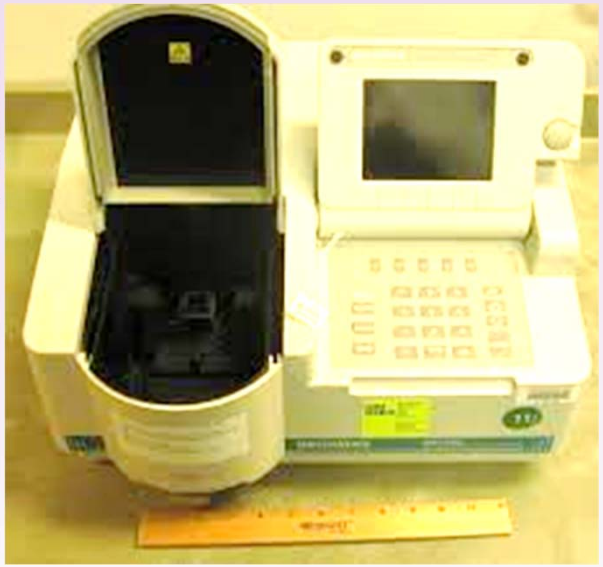
**D**

**PI**

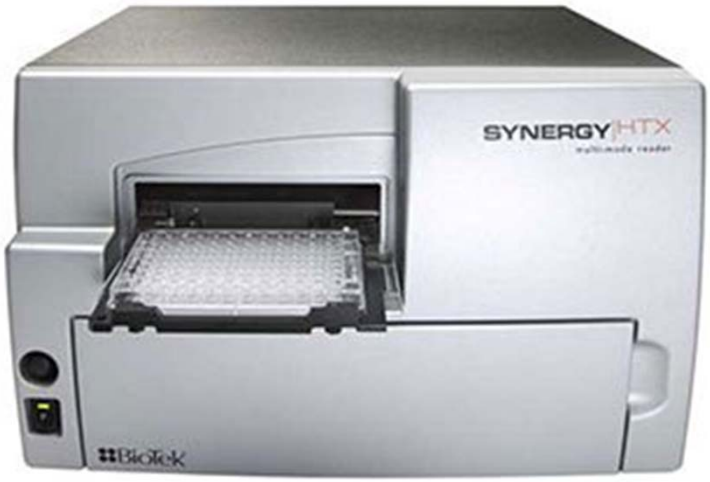


# 1. SPETTROFOTOMETRO

| 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 |

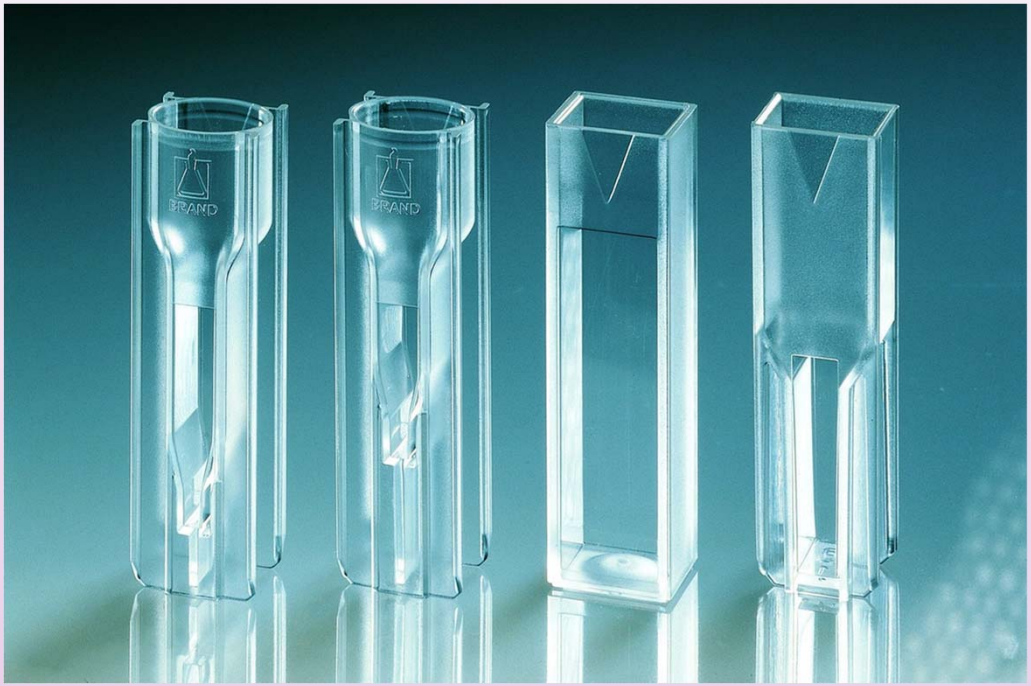



CUVETTA (singolo canale)




PIASTRA

# 2. CUVETTE



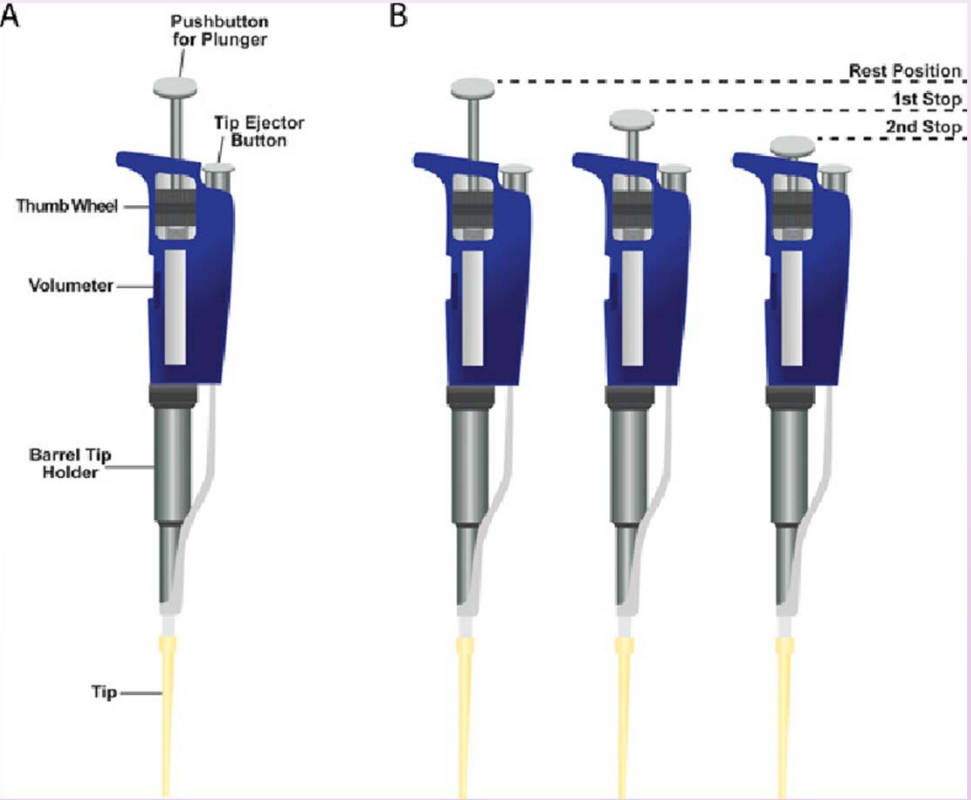

MATERIALE PLASTICO
TRASPARENTE ALLA LUCE VISIBILE.
USA E GETTA

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!

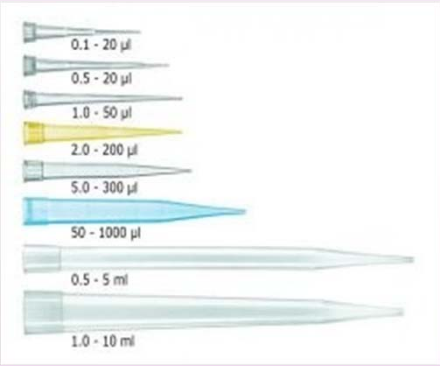
# 3. MICROPIPETTE

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 $cm^3$**

- 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?

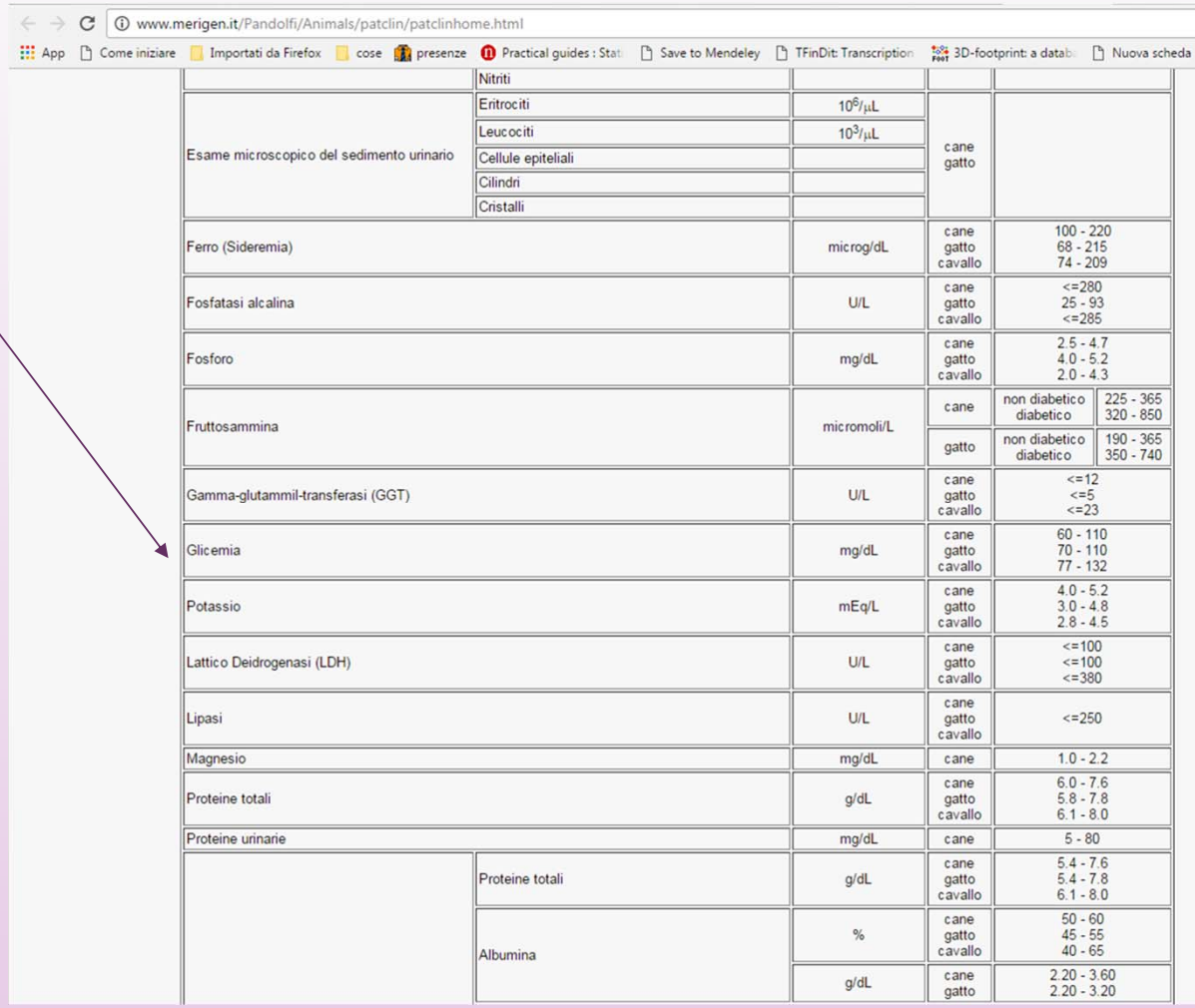
## HK

**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




| | | | | |
|---|---|---|---|---|
| | Nitriti | | | |
| Esame microscopico del sedimento urinario | Eritrociti | $10^6/\mu L$ | cane gatto | |
| | Leucociti | $10^3/\mu L$ | | |
| | Cellule epiteliali | | | |
| | Cilindri | | | |
| | Cristalli | | | |
| Ferro (Sideremia) | | microg/dL | cane gatto cavallo | 100 - 220<br>68 - 215<br>74 - 209 |
| Fosfatasi alcalina | | U/L | cane gatto cavallo | <=280<br>25 - 93<br><=285 |
| Fosforo | | mg/dL | cane gatto cavallo | 2.5 - 4.7<br>4.0 - 5.2<br>2.0 - 4.3 |
| Fruttosammina | | micromoli/L | cane | non diabetico: 225 - 365<br>diabetico: 320 - 850 |
| | | | gatto | non diabetico: 190 - 365<br>diabetico: 350 - 740 |
| Gamma-glutammil-transferasi (GGT) | | U/L | cane gatto cavallo | <=12<br><=5<br><=23 |
| Glicemia | | mg/dL | cane gatto cavallo | 60 - 110<br>70 - 110<br>77 - 132 |
| Potassio | | mEq/L | cane gatto cavallo | 4.0 - 5.2<br>3.0 - 4.8<br>2.8 - 4.5 |
| Lattico Deidrogenasi (LDH) | | U/L | cane gatto cavallo | <=100<br><=100<br><=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<br>5.8 - 7.8<br>6.1 - 8.0 |
| Proteine urinarie | | mg/dL | cane | 5 - 80 |
| | Proteine totali | g/dL | cane gatto cavallo | 5.4 - 7.6<br>5.4 - 7.8<br>6.1 - 8.0 |
| | Albumina | % | cane gatto cavallo | 50 - 60<br>45 - 55<br>40 - 65 |
| | | g/dL | cane gatto | 2.20 - 3.60<br>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 ΔA340 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.

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.

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

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

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

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

$\Delta A = A_{Test} - A_{Total\ Blank}$
TV = Total Assay Volume (mL)
SV = Sample Volume (mL)
Glucose MW = 180.2 g/mole or equivalently
180.2 µg/µmoles
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