## lodide Colorimetric Assay Kit

(Catalog \# K2037-100; 100 assays, Store kit at $-20^{\circ} \mathrm{C}$ )
I. Introduction:
lodide $\left(I^{-}\right)$is a halide anion and a necessary and limiting substrate for thyroid hormone synthesis. This essential element enables the thyroid gland to produce thyroid hormones, thyroxine (T4) and triiodothyronine (T3). Additionally, lodide is important in many essential biological processes such as basal metabolism, temperature regulation, intellectual development for children, muscular development, normal heart function, growth of skeleton etc. Food is the main source of daily supply of iodide in humans. Iodide can be found in grains, fish, dairy products, fruits, iodized salts etc. lodine deficiency affects around two billion people worldwide. lodide deficiency can cause diseases such as goiter, depression, fatigue, hypothyroidism, intellectual disability, delayed sexual development etc. However, excess intake of lodide is also harmful and can inhibit the release of thyroid hormones and cause inflammation of the thyroid gland and even thyroid cancer. BioVision's lodide Colorimetric Assay Kit provides a quick, convenient, non-radioactive method to determine lodide concentration in various sample types including foods, liquid and biological samples. The assay relies on the lodide ion as a specific catalyst to convert a yellow colored substrate to a colorless product. The reduction in absorbance signal at 330 nm is directly proportional to the lodide concentration in the samples. Most common ions (eg. $\mathrm{Na}^{+}, \mathrm{Ca}^{2+}, \mathrm{Cu}^{2+}, \mathrm{K}^{+}, \mathrm{Mg}^{2+}, \mathrm{Fe}^{3+}, \mathrm{NH}^{4+}, \mathrm{CO}_{3}{ }^{2-} \mathrm{Br}^{-}, \mathrm{Cl}^{-}, \mathrm{SO}_{4}{ }^{2-}, \mathrm{NO}_{3}{ }^{-}$and $\mathrm{PO}_{4}{ }^{3-}$ ) do not interfere with the assay. The kit is simple, rapid, sensitive and can detect as low as $0.2 \mu \mathrm{M}$ lodide under the assay conditions.

II. Application:

- Determination of lodide in different samples such as foods, liquid and biological samples
III. Sample Types:
- Foods (e.g. meats, grains, fruits, dairy products etc.)
- Liquids and biological samples (e.g. urine, saliva and serum)
IV. Kit Contents:

| Components | K2037-100 | Cap Code | Part Number |
| :--- | :---: | :---: | :---: |
| lodide Standard | $100 \mu \mathrm{l}$ | Blue | K2037-100-1 |
| lodide Substrate | 1 vial | Yellow | K2037-100-2 |
| Treatment Reagent | 1.1 ml | Red | K2037-100-3 |
| Sample Diluent | 90 ml | NM | K2037-100-4 |
| Precipitation Solution | 5 ml | NM | K2037-100-5 |

V. User Supplied Reagents and Equipment:

- 96-well clear flat-bottom plate
- Multi-well spectrophotometer
- Dounce Tissue Homogenizer (BioVision Cat. \# 1998)
- $\mathrm{ddH}_{2} \mathrm{O}$
VI. Storage Conditions and Reagent Preparations:

Store the kit at $-20^{\circ} \mathrm{C}$. The kit components are stable for one year when stored as recommended. Read the entire protocol before performing the experiment.

- Iodide Standard ( $100 \boldsymbol{\mu M}$ ): Warm to room temperature (RT) before use. Stable at $4^{\circ} \mathrm{C}$ for 2 months.
- Iodide Substrate: Reconstitute the vial with 1 ml of Sample Diluent to prepare the stock lodide Substrate solution. Divide into aliquots and store at $4^{\circ} \mathrm{C}$. Stable for 2 months at $4^{\circ} \mathrm{C}$.
- Treatment Reagent, Sample Diluent \& Precipitation Solution: Ready to use. Warm the bottles to RT before use. Stable for 2 months at $4^{\circ} \mathrm{C}$.
VII. Iodide Assay Protocol:

1. Sample Preparation: For Food Samples: Weigh out $\sim 100 \mathrm{mg}$ of the Sample (e.g. meats, grains, fruits and dairy products etc.) and cut it into small pieces (if possible). Transfer the Sample into an eppendorf tube. Add 0.5 ml of Sample Diluent to the tube and homogenize the Sample for 10 min using dounce tissue homogenizer (BioVision Cat. \# 1998) and incubate at RT for 10 min. Centrifuge the Sample(s) at $12,000 \mathrm{~g}$ for 15 min and collect the clear supernatant. Use $10 \mu \mathrm{l}$ of the clear supernatant for assay.
For Liquid and Urine Samples: Dilute the Sample 2-5 fold using Sample Diluent. Use $10 \mu$ l of the diluted Sample for assay.
For Serum Sample: Add $30 \mu \mathrm{l}$ of Precipitation Solution to $270 \mu \mathrm{l}$ of Serum in an eppendorf tube and vortex briefly. Centrifuge the Sample at $12,000 \mathrm{~g}$ for 15 min and collect the supernatant. Dilute the supernatant 5 fold using Sample Diluent (e.g. $20 \mu \mathrm{l}$ supernatant in $80 \mu \mathrm{l}$ of Sample Diluent). Use $10 \mu \mathrm{l}$ of the diluted supernatant for assay.
2. Standard Curve Preparation: Mix $10 \mu \mathrm{l}$ of $100 \mu \mathrm{M}$ lodide Standard with $990 \mu \mathrm{l}$ of water to prepare $1 \mu \mathrm{M}$ lodide Standard solution. Add $0,2,4,6,8$ and $10 \mu \mathrm{l}$ of $1 \mu \mathrm{M}$ diluted lodide Standard into the desired wells in a 96 -well clear flat-bottom plate to generate $0,0.2,0.4$, $0.6,0.8$ and $1.0 \mu \mathrm{M}$ of lodide Standard/well respectively. Adjust the volume of each well to $10 \mu \mathrm{l}$ using water.
3. Reaction Mix Preparation: Dilute the stock lodide Substrate 10 fold by adding $200 \mu \mathrm{l}$ of stock lodide Substrate solution to 1.8 ml of Sample Diluent (for 20 assays) before performing the assay. Prepare Reaction Mix according to the table below. Make sufficient amount of Reaction Mix to add $90 \mu \mathrm{l}$ to all assay wells.

## Reaction Mix

Diluted Iodide Substrate solution
$80 \mu \mathrm{l}$
Treatment Reagent
$10 \mu \mathrm{l}$

Mix well. Add $90 \mu$ l of Reaction Mix to all the wells containing Standards and Samples.
4. Measurement: Immediately, measure the absorbance (OD) of all wells at 330 nm in kinetic mode at RT for 5 min .
5. Calculation: Subtract the 0 Standard readings from all Standard and Sample readings. For each Standard concentration, subtract the final absorbance ( $t_{2}$ ) from the initial absorbance ( $t_{1}$ ) to get the reduction in absorbance signal ( $t_{1}-t_{2}$ ). Plot the lodide Standard Curve by using the reduction in Absorbance Signal ( $\mathrm{t}_{1}-\mathrm{t}_{2}$ ) values vs lodide Standard concentrations.

Calculate the lodide concentration in the Sample (A) by applying the reduction in Sample Absorbance Signal values to the lodide Standard Curve

$$
\begin{array}{cc} 
& \text { Iodide concentration }(\boldsymbol{\mu M})=\mathbf{A} \times \mathbf{D} \\
\text { Where: } \quad \begin{array}{l}
\text { A }=\text { lodide concentration from the Standard Curve }(\mu M) \\
D=\text { Sample dilution factor }(D=1, \text { for undiluted samples })
\end{array}
\end{array}
$$



B


C


Figures. A. lodide Standard Curve. B. lodide concentration in urine and serum samples before and after spiking with $5 \mu \mathrm{M}$ of iodide. Data shows $>80 \%$ recovery under the assay kit conditions. C. lodide in egg yolk, banana and chocolate samples.

## VIII. Related Products:

Calcium Colorimetric Assay Kit (K380)
Magnesium Colorimetric Assay Kit (K385)
Sodium Assay Kit (Colorimetric) (K391)
Nickel Colorimetric Assay Kit (K510)

Calcium Assay Kit (Fluorometric) (K409)
Iron Colorimetric Assay Kit (K390)
Cobalt Colorimetric Assay Kit (K505)
Potassium (Serum) Detection Assay Kit (Fluorometric) (K940)

