Nonradioactive Iodide Assay kit

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Nonradioactive Iodide Assay kit
#D05076.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed & validated
by Bertin Pharma

Fabriqué en France
Made in France

#D11076
Version: 0117

Table of contents

96 wells
Storage: +4°C
Expiry date: stated on the package

This kit contains:

Designation	Item #	Quantity per kit	Form
Covered 96 well Microtiter plate	D08000.1 ea	1	-
Nonradioactive iodide assay reagent A	D22076.100 dtn	1	Liquid
Nonradioactive iodide assay reagent B	D20076.100 dtn	1	Liquid
Nonradioactive iodide assay standard	D06076.1 ea	1	Liquid
Technical Booklet	D11076.1 ea	1	-
Well cover sheet	-	1	-

Each kit contains sufficient reagents for one 96-well plate. This allows for the construction of one standard curve in duplicate and the assay of 37 samples in duplicate.

Precaution for use

Users are recommended to carefully read all instructions for use before starting work.

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not eat, drink or smoke in area in which kit reagents are handled
- Do not use organic solvent (DMSO, EtOH, MeOH, MeCN, acetone)
- Avoid splashing

The total amount of reagents contains less than 23 mg of arsenic(III) oxide.

Wearing gloves, laboratory coat and glasses is recommended when assaying kit materials and samples.

Waste elimination:
These reagents are dangerous for environment.
- Do not throw in the sink.
- Use appropriate recovery can.

Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), that is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

Iodide background

Present in nature in the form of iodide and iodate, iodine is a solid halogen at normal temperature. It is used in medicine, in the pharmaceutical and food industry. Food is the principal daily supply of iodide in human body ***[1]***.

Iodide is important in basal metabolism and permits temperature regulation, intellectual development for children, muscular development, normal heart function and growth of skeleton. Iodide transport is the basis for an emerging approach of selective cancer cell destruction. ***[2-3]***

Iodide uptake from blood into thyroid follicular cells is the first step in the biosynthesis of thyroid hormones T4 and T3, known to regulate many essential biological processes ***[4-5]***.

Thyroid hormones are indispensable for body development. This transport is mediated by NIS (sodium iodide symporter), an intrinsic membrane glycoprotein located in the basolateral membrane of thyrocytes.

Since the discovery of NIS, thorough biochemical analysis has elucidated the mechanism of basolateral iodide transport and revealed the key role of NIS in thyroid diseases such as thyroid cancer, autoimmune disease, and congenital hypothyroidism ***[6]***.

If rate is not in the normal proportion, some diseases can be developed as underactive thyroid if the rate is too down or overactive thyroid if the rate is too up. Other diseases exist as

chronic thyroiditis of Hashimoto or cancer of the thyroid gland ***[7]***.

Iodide deficiency is at origins of many thyroid metabolism disorders, this is why it is important to control rate of iodide to prevent all of these diseases.

Principle of the assay

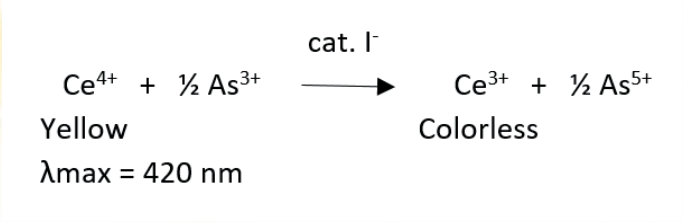
The present assay is a nonradioactive method for the measurement of iodide.

This Iodide Assay is based on the oxido-reduction reaction: cerium(IV) is reduced by arsenic(III). The reduction of yellow (420 nm) cerium(IV) to colorless cerium(III) by arsenic(III) proceeds very slowly but traces of iodide strongly accelerate this reaction with the rate being directly proportional to iodide concentration.

For a given time, decay is inversely proportional to iodide concentration in well.

This method is simple and nonradioactive, and as such it can be used widely.

The principle of the assay is summarized below:



Materials and equipment required

In addition to standard laboratory equipment, the following material is required:

- Precision micropipettes (20 to 1000 µL)
- Spectrophotometer plate reader (414 nm or 420 nm filter)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300µL
- UltraPure water #A07001.1L
- Polypropylene tubes

Water used to prepare all diluted reagents must be UltraPure (deionized & free from organic contaminant traces).
Otherwise, organic contamination can significantly affect the assay.
Do not use distilled water, HPLC-grade water or sterile water.
UltraPure water may be purchased from Bertin Pharma: item #A07001.1L.

Sample collection and preparation

This assay may be used to measure Iodide.

It is the responsability of the user to check the compatibility of the assay with the study matrix. To verify if there is no issue with the desired matrix, compare the data of the standard curve realised in water and in study matrix.

General precautions

- All samples must be free from organic solvents prior to assay.
- Samples should be assayed immediately after collection or should be stored at -20°C.

Reagent preparation

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 37 samples in duplicate.

All reagents need to be brought to room temperature (around +20°C) prior to the assay.

Diluted nonradioactive iodide assay reagent A

The vial #D22076 has to be diluted four times with UltraPure water prior to use.

Use the following formula to determine the total volume for diluted nonradioactive iodide assay reagent A solution required:

(Standards + Samples) x (replicates) x 100µL

Then mix thoroughly by gentle inversion.

Stability: Use within the day

Example: If you want to assay the entire 96-well plate, you need 10mL of diluted nonradioactive iodide assay reagent A:

Dilute 2.5mL of #D22076 in 7.5mL of UltraPure water.

Diluted nonradioactive iodide assay reagent B

Prepare the diluted nonradioactive iodide assay reagent B the same way as reagent A using the vial #D20076.

Diluted nonradioactive iodide assay standards

Dilute 30µL of the vial #D06076 with 2970 µL of UltraPure water; mix thoroughly by gentle inversion.

This 100x diluted solution is then diluted 10x a second time to create the stock solution: pipet 200µL of this solution with 1800 µL of UltraPure water. Then mix thoroughly by gentle inversion.

The concentration of this stock solution is 2 µM.

Prepare eight polypropylene tubes. Then prepare the standards by dilutions as follows:

Standard	Volume of Standard stock solution at 2 µM	Volume of UltraPure water	Standard concentration (nM)
S0	-	1mL	0
S1	350µL	650µL	700
S2	300µL	700µL	600
S3	250µL	750µL	500
S4	200µL	800µL	400
S5	150µL	850µL	300
S6	100µL	900µL	200
S7	50µL	950µL	100

Stability: Use within the day

Assay procedure

It is recommended to perform the assays in duplicate following the instructions hereafter.

Plate set-up

A plate set-up is suggested hereafter for the microplate procedure.
The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bk	S1	S5	*	*	*	*	*	*	*	*	*
B	Bk	S1	S5	*	*	*	*	*	*	*	*	*
C	Bk	S2	S6	*	*	*	*	*	*	*	*	*
D	Bk	S2	S6	*	*	*	*	*	*	*	*	*
E	S0	S3	S7	*	*	*	*	*	*	*	*	*
F	S0	S3	S7	*	*	*	*	*	*	*	*	*
G	S0	S4	*	*	*	*	*	*	*	*	*	*
H	S0	S4	*	*	*	*	*	*	*	*	*	*

BK: Blank S7-S0 : Standards *: Samples

Pipetting the reagents

All samples and reagents must reach room temperature prior to performing the assay.

Use different tips to pipet the buffers, nonradioactive iodide assay reagent A, nonradioactive iodide assay reagent B, standards and samples.

Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expelling with the pipette tip.

- **Blank**
  Dispense 200 µL of water or matrix to prepare the standards to Blank (Bk) wells.

- **Nonradioactive iodide assay Standard**
  Dispense 100 µL of each of the eight standards (So, then S7 to S1) in duplicate to appropriate wells. Start with the lowest concentration standard (S0) and equilibrate the tip in the next higher standard before pipetting (S7 to S1).

- **Nonradioactive iodide assay Samples**
  Dispense 100 µL in duplicate to appropriate wells.

- **Diluted nonradioactive iodide assay Reagent A**
  Dispense 100 µL in each well except Blank (Bk) wells.

- **Diluted nonradioactive iodide assay Reagent B**
  Dispense 100 µL in each well.

Incubating the plate

Cover the plate with the cover sheet and incubate 30 minutes at +20°C in the dark.

Reading the plate

> Wipe the bottom of the plate with a paper towel, and make sure that no liquid has splashed outside the wells.
> Read the plate at a wavelength between 414-420nm (yellow colour).

Protocol in brief			
Volume / Wells	Blank (BK)	Standard	Sample
Standard	-	100µL	-
Sample	-	-	100µL
Diluted reagent A	-	100µL	100µL
Diluted reagent B	100µL	100µL	100µL
Water or Matrix used	200µL	-	-
Cover plate, incubate 30 min at 20 °C in dark			
Read the plate at 414-420nm			

Data analysis

Make sure that your plate reader has subtracted the absorbance readings of the blank well from the absorbance readings of the rest of the plate. If not, do it now.

- Calculate the average absorbance for each standard and sample.
- Calculate the log of each absorbance (log A).
- For each standard, using a semi-log graph, plot the log A on y axis versus the concentration on x axis
- Use a linear regression.
- Calculate r^2 of your curve.
- To determine the concentration of your sample, find the absorbance value of each sample on the y axis.
- Read the corresponding value on the x axis which is the concentration of your unknown sample.
- Samples with a concentration greater than 700 nM should be re-assayed after dilution.

Acceptable range

- Regression coefficient r^2 >0.98
- Absorbance of S0 ≥ 0.800 A.U

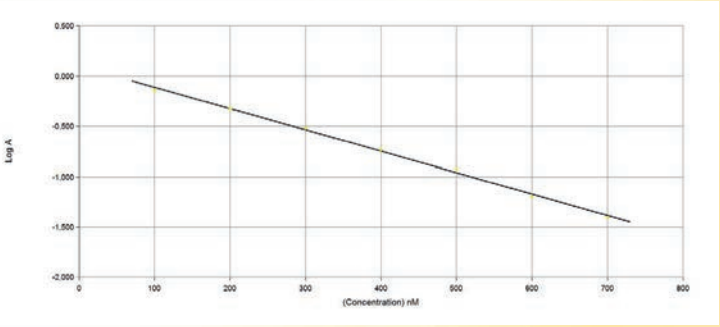
Typical results

The following data are for demonstration purpose only. Your data may be different and still correct.

These data were obtained using all reagents as supplied in this kit under the following conditions: 30 minutes developing at +20°C, reading at 414 nm. A linear regression fitting was used to determine the concentrations..

Standard	Expected concentration (nM)	Log A	Absorbance A.U
S1	700	-1.468	0.040
S2	600	-1.229	0.064
S3	500	-0.979	0.119
S4	400	-0.761	0.184
S5	300	-0.509	0.301
S6	200	-0.282	0.473
S7	100	-0.12	0.728

Typical Nonradioactive iodide standard curve



Troubleshooting

> **Absorbance values are too low**:
- organic contamination of water,
- one reagent has not been dispensed,
- incorrect preparation / dilution,
- assay performed before reagents reached room temperature,
- incubation in wrong conditions (time or temperature).

> **High signal and background in all wells**:
- high ambient temperature.

> **High dispersion of duplicates**:
- poor pipetting technique.

These are a few examples of troubleshooting that may occur.

If you need further explanation, Bertin Pharma will be happy to assist you. Feel free to contact our technical support staff by phone (+33 (0)139 306 036), fax (+33 (0)139 306 299) or E-mail (bioreagent@bertinpharma.com), and be sure to indicate the batch number of the kit (see outside the box).

Bibliography

Additional readings

List of publications quoting the use of this kit

1. Blazy P.
   Iode
   El-Aid JDID, 10 juin 2009

2. Role of Iodine in Metabolism.
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3. Spitzweg C., Morris J. C.
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   The role of thyroid hormones in prenatal and neonatal neurological development,
   Endoc.Rev, 1993, pages 94-106

5. Anna Milanesi, Gregory A.Brent
   Iodine and thyroid hormone synthesis, metabolism, and action.
   Molecular genetics and nutritional aspects of major and trace minerals, 2017, pages 143-150

6. Waltz F., Pillette L., Ambroise Y.
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   Analytical Biochemistry,16 Juillet 2009

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Thyroid Hormone transport and transporters,
Vitamins and hormones ,12 june 2017

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