Iodine HPLC Assay

Catalog Number: IOD34-H100
100 Tests
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 866-419-2019 Fax: 617-419-1110
www.EagleBio.com

1. Intended purpose

The Eagle Biosciences Iodine HPLC Assay kit is intended for the quantitative determination of iodine in urine. The Iodine HPLC Assay is for research use only and is not for use in diagnostic procedures.

2. Introduction

Iodine is an essential mineral which must be ingested by nutrition. The thyroid hormones T3 (triiodidethyronine) and T4 (tyroxine) are built by the addition of iodine to the amino acid tyrosine. T3 is much more effective than T4. T3 is taken up into the cells and binds to receptors of the nucleus. T3 activates a lot of metabolic pathways. It increases body temperature and oxygen consumption. T3 accelerates the ingestion of carbohydrates, activates the release of fat, and supports the turnover of cholesterol and the protein synthesis. It regulates the water balance, the bone metabolism and is indispensable for the maturation of skeleton and brain.

The measurement of free iodine in urine is a valuable tool for the determination of the supply with iodine. The measurement of the direct intake of iodine is rather difficult. Therefore the determination of iodine in urine is well accepted as an index of iodine uptake. The fecal excretion is negligible.

The Eagle Biosciences Iodine HPLC Assay kit makes it possible to determine iodine levels in an easy, fast and precise method. The Iodine HPLC Assay includes all reagents in a ready to use form for preparation and separation of the samples with exception of the columns (IC3100rp) and the controls (IC3100ko). Both can be supplied by Eagle Biosciences. Beside the complete test kits it is possible to order all components separately. Please request our single component price list.

3. Warnings and precautions

- All reagents of this Iodine HPLC Assay kit are strictly intended for research use only.
- The Iodine HPLC Assay kit and column are concerted. Using alternative columns might cause in insufficient separation, resulting in false high results. The given test characteristics might not be fulfilled.
- Do not interchange the Eagle Biosciences Iodine HPLC Assay kit components from different lots.
- Calibrator and controls contain human serum. It was tested and found negative for HBsAg, anti-HIV-1/2, and anti-HCV. No test can guarantee the absence of

HBsAg or HIV, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.

- Wear disposable gloves while handling specimens or Iodine HPLC Assay kit reagents and wash hands thoroughly afterwards.
- Do not pipette by mouth.
- The mobile phase contains acetonitrile and has to be handled carefully. Acetonitrile is highly flammable and toxic if inhaled or contact the skin. It should be handled with gloves, eye protection, and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician.
- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
- Iodine HPLC Assay kit reagents should not be used beyond the expiration date shown on kit label.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

4. Materials Provided

Article no.	Component	Designation	Amount
IC3100lm	ELU	Mobile phase	1000 ml
IC3100st	STD	Standard (1 ml; concentration is given on the label)	1 vials
IC3100c2	COND2	Conditioning solution 2	300 ml

5. Additional Special Equipment

- Vortex mixer
- Various pipettes
- Centrifuge
- HPLC with electrochemical-detector and silver electrode
- HPLC column Iodine (IC3100rp)
- SPE cartridges (IC3100ck)

6. Reagent preparation

- Reconstitute the **calibrator (CAL)** in **3 ml** deionized water, divide the calibrator in several portions and store them at -20 °C. Avoid repeated freeze-thaw circles. The concentration of iodine might have minor changes from lot to lot.
- All test reagents of the Iodine HPLC Assay kit are stable at 20-25 °C, the calibrator at -20 °C up to the date of expiry stated on the label.

7. Specimen

- Urine could be used in the Iodine HPLC Assay kit.
- Samples are stable for 24h at room temperature and up to one week at 2-8 °C. For longer storage the samples should be kept at -20°C.

8. Procedure

Principle of the method

For the determination of free iodine the samples are extracted on solid phase extraction cartridges. The probe is centrifuged and 50 µl of the supernatant are injected into the HPLC system. The isocratic separation via HPLC at 30°C uses a "reversed phase"column. One run lasts 10 minutes. The chromatograms are recorded by an electrochemical detector with a silver working electrode. The quantification is performed with the delivered standard; the concentration is calculated via integration of the peak areas or heights.

Sample preparation

1. The cartridge is rinsed with 3 ml methanol and 3 ml COND2

   Important notice: We highly recommend not to use a centrifuge for rinsing the SPE cartridge with methanol. Methanol is highly flammable and might cause an explosion.

2. **2 ml** sample, CAL or CTRL are soaked through the column (alternatively a centrifuge can be used 4 min at 500 rpm). Discard the break-through.

3. **1 ml** sample, CAL or CTRL are soaked through the column (alternatively a centrifuge can be used 4 min at 500 rpm).

4. Centrifuge the eluate and inject 50 µl in the HPLC system.

Chromatographic settings

Column material:	Iodine HPLC column (IC3100rp)
Flow rate:	1 ml/min
Electrochemical detection:	Ag working electrode Ework: 0.1 V
Injection volume:	50 µl
Running time:	10 min
Temperature:	30 °C

Treatment of the HPLC column

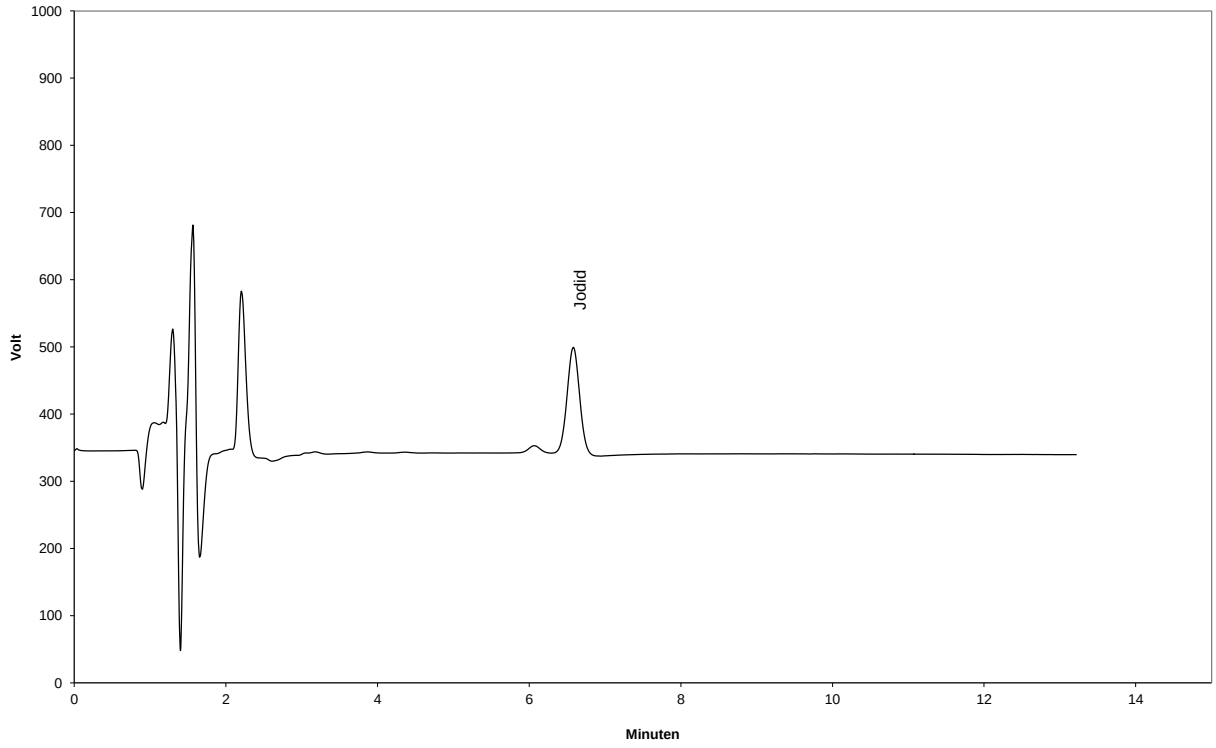
After the analysis the column should be flushed with 15 ml deionized water. (1 ml/min) and stored in 50% methanol in deionized water (ca. 15 ml, flow 0.7 ml/min). Before use, the system should be equilibrated with approx. 30 ml eluent.

9. Calculation of analytical results

Calculation

$$\text{Conc. sample} = \frac{\text{peak area patient} * \text{conc. calibrator}}{\text{peak area calibrator}}$$

Typical chromatogram



10. Validation data

Precision and reproducibility

Intra-Assay VK:	3.5 % (0.53 µmol/l)	[n = 6]
	3.2 % (2.62 µmol/l)	[n = 6]
Inter-Assay VK:	3.5 % (0.49 µmol/l)	[n = 6]
	4.8 % (2.05 µmol/l)	[n = 6]

Linearity

up to 3 pmol/l

Detection limit

0.02 µmol/l

Recovery

99.3 %

11. Limitations of the method

Serum, plasma or whole blood should not be used in the Iodine HPLC Assay kit.

12. Disposal

The mobile phase (ELU) must be disposed as non-halogenated solvent. Please refer to the appropriate national guidelines.

13. Troubleshooting

Problem	Possible reason	Solution
No signal	No or defect connection to evaluation system	Check signal cord and connection
	Detector lamp is altered	Change lamp
No peaks	Injector is congested	Check Injector
Double peaks	Dead volume in fittings and / or column	Renew fittings and / or column
Contaminating peaks	Injector dirty	Clean injector
	Contamination at the head of the column	Change direction of the column and rinse for 30 min at low flow rate (0.2 ml/min) with mobile phase
	Air in the system	Degas pump
	Autosampler vials contaminated	Use new vials or clean them with methanol
Broad peaks, tailing	Precolumn / column exhausted	Use new precolumn / column
Variable retention times	Drift in temperature	Use a column oven
	Pump delivers imprecise	Check pump, degas the system
	System is not in steady state yet	Rinse system mobile phase for 15 min
Baseline is drifting	Detector lamp did not reach working temperature yet	Wait
	Detector lamp is too old	Renew lamp
Continue baseline is drifting	System is not in steady state yet	Rinse system mobile phase for 15 min
	Pump delivers imprecise	Check pump, degas the system
Baseline is not smooth	Pump delivers imprecise	Check pump, degas the system
	Detector flowcell is dirty	Clean flow cell

For further information about this kit, its application or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.