HiPer® RNA Estimation Teaching Kit

Product Code: HTBC007

Number of experiments that can be performed: 5/20

Duration of Experiment

Protocol: 1 hour

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Store RNA Standard and RNA Samples at -20°C
- Store Diluent Buffer, Orcinol monohydrate and Ferric chloride anhydrous at room temperature (15-25°C)

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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Aim:

To determine the concentration of RNA by orcinol method

Introduction:

HiPer® RNA Estimation Teaching Kit is designed for rapid and accurate determination of RNA by orcinol reagent. This method depends on the conversion of pentose in the presence of hot acid to furfural which then reacts with orcinol to yield a green colour. The colour intensity can be measured at 665 nm.

Principle:

Among the various colorimetric methods for RNA estimation, the orcinol reaction of Mejbaum as modified by Schneider is the most sensitive method for purine-bound ribose quantitation. In this method, RNA is depurinated in concentrated HCl and the resulting ribosephosphates are dephosphorylated and dehydrated to produce furfural. Furfural then reacts with orcinol in the presence of $Fe^{3+}$to yield colored condensation products, which together possess an absorption maximum at 660 nm. The colour intensity is measured at 665 nm which is directly proportional to the concentration of RNA.

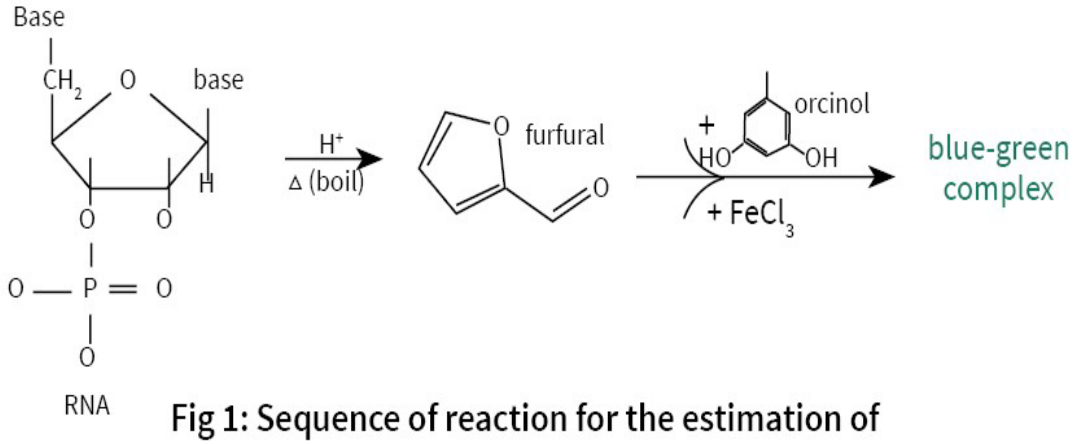


Fig 1: Sequence of reaction for the estimation of RNA by orcinol method

Kit Contents:

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity		Storage
			5 expts	20 expts	
1	TKC221	RNA Standard (1mg/ml)	3.5 ml	14 ml	-20°C
2	RM460	Orcinol, monohydrate	0.36 g	1.44 g	RT
3	GRM1178	Ferric chloride, anhydrous	0.15 g	0.6 g	RT
4	ML030	20X SSC	0.4 ml	1.2 ml	RT

5	TKC208	RNA Sample 1	1.2 ml	4.8 ml	-20°C
6	TKC209	RNA Sample 2	1.2 ml	4.8 ml	-20°C

Materials Required But Not Provided:

Glasswares: 1 ml, 10 ml Pipettes, cuvettes, test tubes

Reagents: Ethanol (95 – 100%), Concentrated HCl, Distilled Water*

Other requirements: Spectrophotometer/Colorimeter to determine the absorbance at 665 nm, Micropipette and tips, Boiling water bath

***Recommended product for use: ML064 – Molecular Biology Grade Water**

Storage:

HiPer® RNA Estimation Teaching Kit is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store RNA Standard, RNA Sample 1 and RNA Sample 2 at -20°C. Orcinol, 20X SSC and Ferric chloride powder can be stored at room temperature.

Important Instructions:

- Read the entire procedure carefully before starting the experiment.
- All glasswares should be clean and detergent free, otherwise it will interfere with the assay.
- The unknown and standard samples should be treated identically for accurate results.
- The assay should be carried out at the same time and in the same buffer conditions.
- **Preparation of Orcinol Reagent (25 ml)**: Dissolve 0.025 g of $FeCl_3$ in 25 ml of Conc. HCl. Add 875 µl of 6% Orcinol in ethanol to it. The reagent should be prepared freshly.
- **Preparation of 6% Orcinol Solution**: Dissolve 0.36 g of Orcinol in 6 ml of ethanol and store at RT in amber colored bottle.
- **Preparation of 1X SSC (Saline Sodium Citrate) (6 ml):** Add 300 µl of 20X SSC to 5.7 ml of distilled water.

Procedure:

1. Make dilutions of RNA standard with concentrations of 40, 80, 120, 160, 200 µg/ 200 µl by transferring respective amount of RNA from the standard RNA solution (1 mg/ml) and adjusting it to a total volume of 200 µl by adding 1X SSC as a diluent buffer as mentioned below.
2. Add 3 ml of freshly prepared Orcinol Reagent to each test tube including the Blank and Unknown tubes. Mix well.
3. Keep it in Boiling Water Bath for 20 minutes and cool.
4. Switch on the Spectrophotometer, select the wavelength at 665 nm and let it warm before taking the absorbance (OD). First take the OD of Blank and make it zero.
5. Remove Blank tube and take the OD of all the tubes and record it. Wash the cuvette after taking OD of each sample.

NOTE– If the developed colour is dark green (absorbance is more than 1.0), dilute the solution and take the O.D

Tube No.	Blank	1	2	3	4	5	6	7
Conc. of RNA (µg)	0.0	40	80	120	160	200	RNA sample 1	RNA sample 2
Amt of Stock (µl)	0.0	40	80	120	160	200	200 µl	200 µl
Amt of 1X SSC (µl)	200	160	120	80	40	0.0		
Amt of Orcinol reagent (ml)	3	3	3	3	3	3	3	3
Keep in Boiling water bath for 20 minutes and then cool down to room temperature								
Absorbance at 665 nm								

6. Plot a Standard Curve of absorbance at 665 nm on "Y" axis versus concentration of RNA µg/200µl on "X" axis.

7. Record the value "x" of Unknown from graph corresponding to the optical density reading of the test.

Determination of RNA Concentration in Unknown Sample:

RNA concentration in unknown sample can be calculated using the following formula:

$$\text{RNA Concentration in Unknown Sample} = \frac{\text{Concentration of Unknown in "µg"}}{\text{Volume of sample in "µl"}} \times 5$$

$$= \_\_\_\_\_ \text{ µg/ml}$$

Observation and Result:

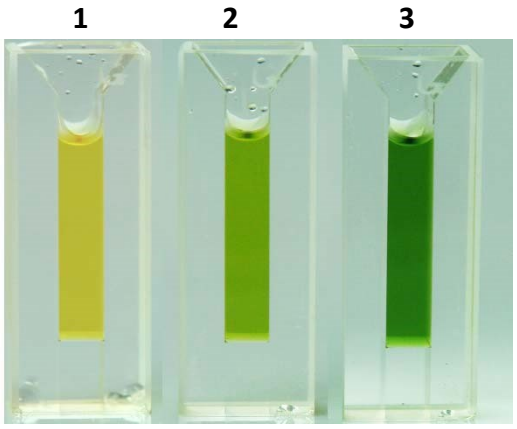
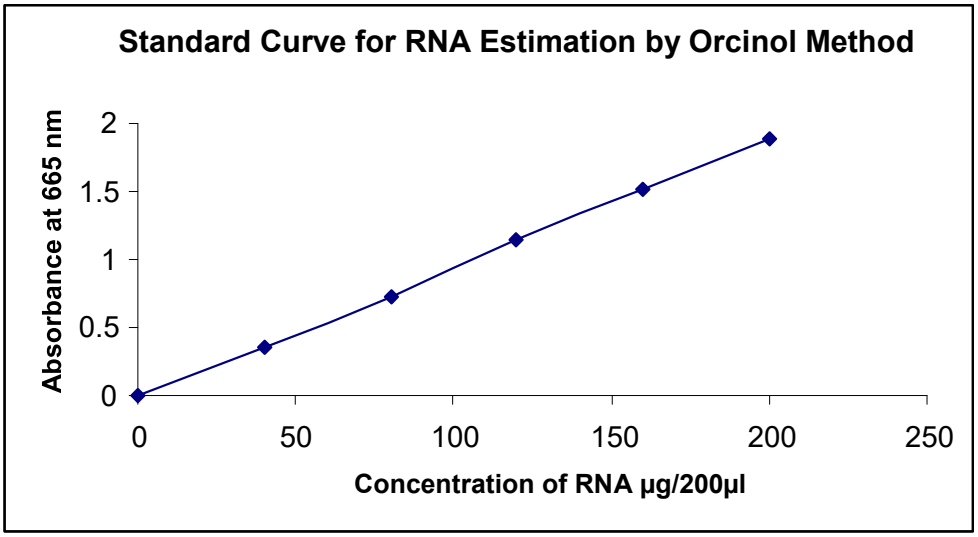


Fig 2: RNA Estimation by Orcinol method – showing increasing amounts of RNA concentration



The absorbance of the RNA solution at 665 nm increases with increasing RNA concentration

Interpretation:

The Orcinol method is carried out by preparing a set of solutions with known RNA concentrations and mixing them with the Orcinol reagent. A standard curve can be made and the concentrations of unknown RNA sample can be derived from the standard curve.

Trouble shooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1	Standards and Samples give lower OD values than expected although the Blank is ok	Orcinol reagent was not prepared freshly	Prepare Orcinol reagent freshly, prior to use
		Absorbance was not measured at correct wavelength	Measure absorbance at correct wavelength as mentioned in the brochure

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of Technical assistance mail at mb@himedialabs.com.

Symbol:

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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