

Estimation of RNA by Orcinol Reaction

Dr. Mahesha H B, Yuvaraja’s College, University of Mysore, Mysore.

Aim: To estimate the concentration of RNA by orcinol reaction.

Principle: This is a general reaction for pentoses and depends on the formation of furfural when the pentose is heated with concentrated hydrochloric acid. Orcinol reacts with the furfural in the presence of ferric chloride as a catalyst to give a green colour, which can be measured at 665 nm.

Requirements:

- 1. Standard RNA solution- 200µg/ml in 1 N perchloric acid/buffered saline.
- 2. Orcinol Reagent- Dissolve 0.1g of ferric chloride in 100 ml of concentrated HCl and add 3.5 ml of 6% w/v orcinol in alcohol.
- 3. Buffered Saline- 0.5 mol/litre NaCl; 0.015 mol/litre sodium citrate, pH 7.

Procedure:

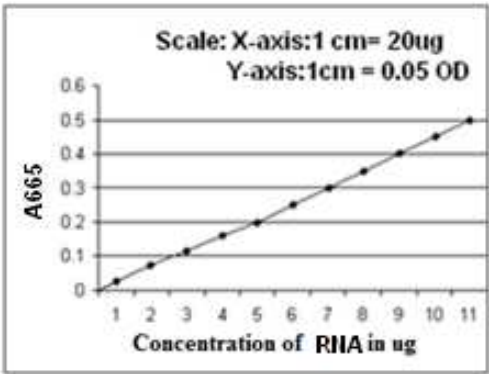
- 1. Pipette out 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labeled test tubes.
- 2. Pipette out 1 ml of the given sample in another test tube.
- 3. Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water serves as the blank.
- 4. Now add 2 ml of orcinol reagent to all the test tubes including the test tubes labeled 'blank' and 'unknown'.
- 5. Mix the contents of the tubes by vortexing / shaking the tubes and heat on a boiling water bath for 20 min.
- 6. Then cool the contents and record the absorbance at 665 nm against blank.
- 7. Then plot the standard curve by taking concentration of RNA along X-axis and absorbance at 665 nm along Y-axis.
- 8. Then from this standard curve calculate the concentration of RNA in the given sample.

Result: The given unknown sample contains ----µg RNA/ml.

Observations and Calculations

Volume of standard (200 µg/ml) RNA	Volume of distilled water (ml)	Concentration of RNA (µg)	Volume of Orcinol reagent (ml)	Incubate in boiling water bath for 20 Min & Cool	A ₆₆₅
0.0	1.0	00	2		0.00
0.2	0.8	40	2		
0.4	0.6	80	2		
0.6	0.4	120	2		
0.8	0.2	160	2		
1.0	0.0	200	2		
1.0 Unknown	0.0	To be estimated	2		

Standard curve for RIA estimation by Orcinol reaction



References

1. Plummer D.T., 1988. An introduction Practical Biochemistry, 3rd ed, Tata Mc Graw-Hill Pub.Co., New Delhi, pp 221.

2. Introductory Practical Biochemistry, Editors: S.K.Sawhney & Randhir Singh, Narosa Pub. House, pp 74-75.
