

# Aim 2

## To Estimate the Amount of RNA in given Sample

### Introduction

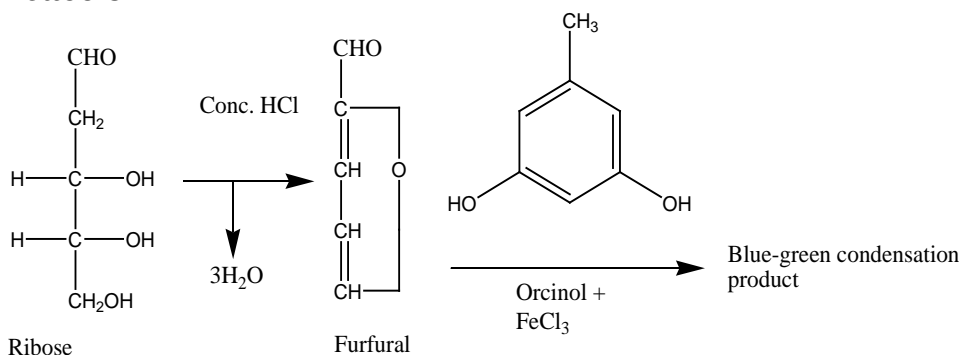
In acidic condition ribose sugar of RNA undergoes dehydration and forms furfural which on reaction with orcinol in presence of ferric chloride yields blue-green compound. This reaction is generally carried out for pentose sugar.

Orcinol reacts with ribose attached with the purine nucleotides and gives the value that represents half of the total ribose.

### Reagents

1. Saline buffer (pH 7.0, 0.015 M sodium citrate and 0.15 M NaCl)
2. RNA solution (10 mg/10 ml in saline buffer)
3. Orcinol reagent (1 g of ferric chloride is dissolved in 1 L of Conc. HCl, then add 35 ml of 6 % w/v orcinol in alcohol to it).

### Reaction



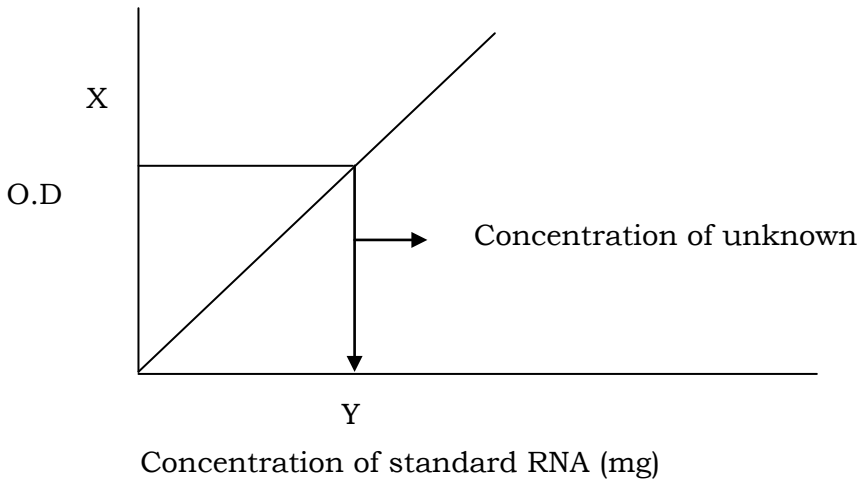
### Procedure

1. The different volume of standard RNA solutions is pipetted out in a series of different test tubes.
2. 0.5 ml and 1.0 ml of test sample is pipetted out in test tubes.
3. One test tube is taken as blank.
4. Add saline buffer as given in observation table to make final volume 2 ml.
5. 3 ml of orcinol reagent is added to each test tube to make final volume to 5 ml.
6. Put all the test tubes in a boiling water bath for 20 minutes.
7. Cool and take the O.D. at 660 nm against blank.

### Observation table

S.No.	RNA volume (ml)	RNA Conc. (mg)	Buffer volume (ml)	Orcinol reagent (ml)		O.D. 660 nm
Blank	-	-	2.0	3	Put it on water bath for 20 minutes	
1	0.2	0.2	1.8			
2	0.4	0.4	1.6			
3	0.6	0.6	1.4			
4	0.8	0.8	1.2			
5	1.0	1.0	1.0			
6	1.2	1.2	0.8			
7	1.4	1.4	0.6			
8	1.6	1.6	0.4			
9	1.8	1.8	0.2			
10	2.0	2.0	-			
Unknown	0.5		1.0			
	1.0		-			

**Standard curve**



**Calculations**

O.D of taken sample - X

From standard curve

Suppose X O.D. corresponds to y mg of RNA

Test sample taken – 0.5 ml

So 0.5 ml of the unknown sample contains Y mg of RNA.

$$\text{RNA (mg \%)} = Y/0.5 \times 100$$

Alternatively, the concentration can be calculated by the formula.

O.D. test -.....

O.D. Standard -.....

$$\text{RNA (mg \%)} = (\text{O.D. test} / \text{O.D. standard} \times \text{concentration of standard} / \text{volume of sample in ml}) \times 100$$

The value resulted by reaction of ribose of purine nucleotides, represents half of the total ribose in the sample. Actual value can be obtained by multiplying RNA concentration with 2.

**Precautions**

1. All apparatus should be clean and dry.
2. Fresh orcinol reagent should be used.
3. RNA sample should be weighed accurately.
4. The solution should be carefully pipetted out.
5. Do not pipette orcinol reagent via mouth as its fumes are harmful.