

Fluorometric Assay of RIBOFLAVIN BINDING PROTEIN, Apo Form

PRINCIPLE:

RBP + Riboflavin (Fluorescent) \longrightarrow RBP-Riboflavin Complex (Quenched)

Abbreviations used:

RBP = Riboflavin Binding Protein

CONDITIONS:

Excitation = 450 nm, Emission = 530 nm, Light path = 1 cm,
T = 25°C, pH = 5.5

METHOD: Fluorimetric Determination

REAGENTS:

- A. 40 ug/ml Riboflavin Stock Solution
(Prepare 500 ml in deionized water containing 1 ml of concentrated HCl using Riboflavin, Prod. No. R-4500.)
- B. 1.0 ug/ml Riboflavin Solution
(Prepare 40 ml in deionized water using Reagent A.
PREPARE FRESH.)
- C. 50 mM Sodium Acetate Buffer pH 5.5 at 25°C
(Prepare 50 ml in deionized water using Acetic Acid, Glacial, Prod. No. A-0808 and adjust pH to 5.5 with NaOH.)
- D. Riboflavin-Binding Protein Solution
(Prepare a solution containing 1 mg/ml in cold deionized water.)
- E. 1% (w/v) Bovine Serum Albumin (BSA Solution)
(Prepare 100 ml in deionized water using Albumin, Bovine, Prod. No. A-4503.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable tubes.

	<u>Blank</u>	<u>Control</u>	<u>Sample</u>
Reagent E (BSA Solution)	1.00	1.00	1.00
Reagent D (RBP)	-----	-----	0.050
Reagent B (Riboflavin Solution)	-----	1.00	1.00
Deionized Water	1.00	-----	-----
Reagent C (Sodium Acetate Buffer)	10.00	10.00	10.00

Transfer solutions into suitable cuvettes. Zero the fluorimeter on the Blank cuvette. Read the excitation of the Control and Sample.

CALCULATIONS:

F_1 = Fluorescence reading of Sample

F_2 = Fluorescence reading of Control

If $F_1/F_2 < 0.1$, sample must be diluted.

$\mu\text{g Riboflavin/mg Protein} = [1 - (F_1/F_2)] / (\text{mg Protein/RM})$

RM = Reaction mix

BINDING CAPACITY:

Expressed as $\mu\text{g riboflavin bound per mg protein}$.

FINAL ASSAY CONCENTRATIONS:

In a 12.0 ml reaction mix the final concentrations are 0.083% Bovine Serum Albumin, 4.2 $\mu\text{g/ml}$ Riboflavin Binding Protein, 0.083 $\mu\text{g/ml}$ Riboflavin, and 42 mM Sodium Acetate.

REFERENCES:

Farrell *et. al.*, (1969) *BBA*, **194**, 432-442.

NOTES:

1. All products and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.