

UNKNOWN _____**DATE** _____**STOCK SOLUTIONS:**

2% Na ₂ CO ₃ in 0.1 N NaOH + 1 % SDS	10 ml
1 % Na tartrate	0.1 ml
1 % CuSO ₄	0.1 ml

BSA standard (high quality expensive BSA) 200 µg/ml in H₂OFolin-Ciocalteu Phenol Reagent (Sigma F-9252)
dilute 1:1 with H₂O

TUBE	BSA µl	H ₂ O µl	MIX		1:1 DILN FOLIN		OD ₇₅₀	µg/ml
1	-	50	0.5 ml	10' RT	50 µl	30'RT		blank
2	-	50	0.5 ml	10' RT	50 µl	30'RT		blank
3	5	45	0.5 ml	10' RT	50 µl	30'RT	_____	1.66
4	10	40	0.5 ml	10' RT	50 µl	30'RT	_____	3.33
5	20	30	0.5 ml	10' RT	50 µl	30'RT	_____	6.67
6	30	20	0.5 ml	10' RT	50 µl	30'RT	_____	10
7	40	10	0.5 ml	10' RT	50 µl	30'RT	_____	13.33
8	50	-	0.5 ml	10' RT	50 µl	30'RT	_____	16.66

TUBE	UNKNOWN 1	H ₂ O	OD ₇₅₀
—	5	45	_____
—	10	40	_____
—	20	30	_____
—	30	20	_____
—	40	10	_____
—	50	-	_____

TUBE	UNKNOWN 2	H ₂ O	OD ₇₅₀
—	5	45	_____
—	10	40	_____
—	20	30	_____
—	30	20	_____
—	40	10	_____
—	50	-	_____

Read A₇₅₀ (or A₅₀₀ if too strong).Plot A₇₅₀ vs µg/ml for the BSA standard.Locate A₇₅₀ of unknown on curve ⇒ read out corresponding µg/ml.

The total assay volume = 600 µl ⇒ to get the protein concentration of the unknown, multiply the value extrapolated from the line by 600/x, where x is the number of µl of unknown added to give that A₇₅₀.