QUIZ 3 MAY 12, 2017
MCB 120L S2017V2

Name:_____

Lab Section (circle): **MORAND A01** **LAGARIAS A02**

Acid phosphatase (APase) is an enzyme that, like alkaline phosphatase studied in Exp. 3, can catalyze p-nitrophenol phosphate hydrolysis. You have been asked to determine the specific activity of APase in an extract from the newly isolated archeabacterium *Thermococcus furiosus*. Since you have taken MCB120L, you know that you need to determine both **protein concentration** and **APase enzyme activity** of the *T. furiosus* extract. The total volume of the extract is 2.1 ml.

T. furiosus cells are hard to obtain in quantity since it only grows near volcanic vents deep in the ocean. For this reason, you used a Micro BCA Protein Assay in which BSA standard and Babelfish extracts were diluted into 50 µl (final volume) from which 12.5 µl was removed and diluted with 237.5 µl BCA reagent in individual wells of a microplate. This colorimetric assay measures the change in absorbance at 562 nm. You analyzed different amounts of your undiluted *T. furiosus* extract and obtained the results shown in Table 1 below. The BCA standard curve that uses BSA as the protein standard is shown below on the right. The formula for the best-fit line of this data is **y = 0.0554x + 0.488** where y corresponds to A_{562} and x corresponds to µg protein.

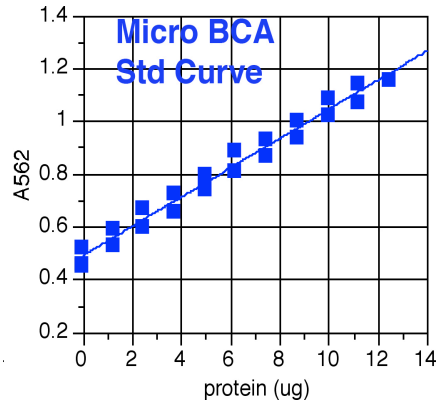

Table 1. BCA Protein Assay

Sample Mixtures		**Assay Results**	
APase extract assayed, µl	Amount of water added, µl	Ave A_{562} for 12.5 µl sample mix + 237.5 µl BCA reagent (n=3)	µg protein in assay
2	48	0.500	
4	46	0.712	
8	42	0.942	
16	34	1.282	

1. (2 pts) Complete the last column of data table above. In the box below, indicate the **concentration** of APase protein in your *T. furiosus* extract (mg/ml) and the **total protein** (mg) in the *T. furiosus* extract. Show your work below or on back.

Conc =

Total Protein =

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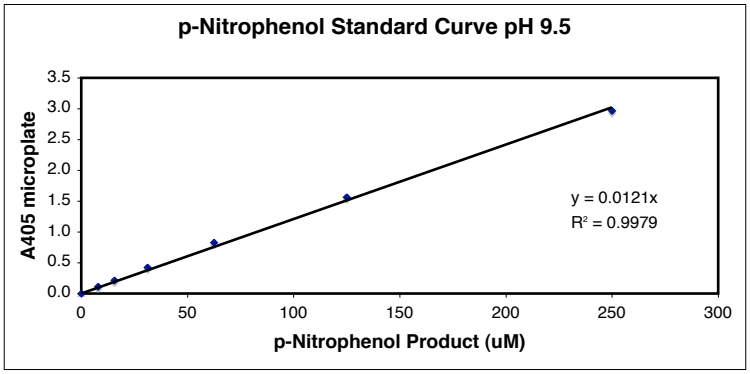
You next assayed the *T. furiosus* extract for APase activity at four pHs using the **continuous** and microplate **fixed time** assays protocol **identical** to those you used in Experiment 3A&B, **except that the fixed time was shortened to 4 min.** The assay results for **1/50 dilutions** of the *T. furiosus* extract are shown below. Since acidic buffers were used for these assays, the pH of the fixed time assays were measured after the 1.0 M K_2HPO_4 Stop solution was added to permit correction for the ionization of p-nitrophenol product in the microplate. This was done with a micro pH electrode and the pHs measured are shown in the Table. The standard curve for p-nitrophenol for the microplate is shown below.

Continuous Assay (1 cm cuvette)					**Microplate Fixed Time Assay**					
Assay pH	**Non-enz. control ΔA_{410} min^{-1}**	**+ Enz. ΔA_{410} min^{-1}**	**Corr. ΔA_{410} min^{-1}**	**I.U. per assay**	**Assay pH**	**Measured pH after 1.0 M K_2HPO_4 Stop solution added**	**Non-enz. ΔA_{410} $(4\ min)^{-1}$**	**+ Enz. ΔA_{410} $(4\ min)^{-1}$**	**Corr. ΔA_{410} $(4\ min)^{-1}$**	**I.U. per assay**
4	0.0003	0.0005	0.0002	**XXX**	**4**	7.0	0.010	0.4765		
5	0.0002	0.0020	0.0018	**XXX**	**5**	7.5	0.050	0.6980		
6	0.0001	0.0100	0.0099	**XXX**	**6**	8.0	0.080	0.5728		
7	0.0002	0.0753	0.0751		**7**	8.5	0.102	0.4609		



2. (3 pts) Based on the continuous assay results, calculate the specific activity of the *T. furiosus* APase at **pH 7**. Put IU per assy in Table above. Show how you calculated this value below and place your calculated specific activity in the box below.

APase sp. activity @ pH 7 =

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3. (4 pts) Complete the Table on the previous page. In the space below (and continued on next page or back), show how you calculated the I.U. per assay for the **fixed time** assays measured at pH 7. Put answers in Table on page 1. All other calculations can be performed on back pages.

4. (1 pt) Based on your data (above), at which pH is the activity of APase the largest – **pH 4**, **pH 5**, **pH 6** or **pH 7** (CIRCLE ANSWER)? Justify your answer below.