

Time: TU 8:00 - 11:00 & 13:00 - 16:00/ TH 9:00 - 12:00

Room: 504

Topic	Lecture	Period	Date	Instructor	LAB	Period	Date	1st Instructor	2nd Instructor	3rd Instructor	4th Instructor
Overall biochemistry lab safety Report writing		1	7 Jan (A)	SJ							
1. Cell feactionation, enzyme purification and characterization								Nuchanat	Manchumas	Veerasak	Supitcha
1.1 Concept of enzyme purification and characterization	Concept of enzyme purification and characterization	1	9-Jan	NW							
1.2 Reagent and buffer preparation	pH and buffer	1	14 (M) Jan	RP	Reagent and buffer preparation	2	14 (A), 16 Jan				
1.3 Cell lysis and Differential fractionation	1. Cell lysis and cell fractionation (Differential centrifugation)	1	21 (M) Jan	SW	1. Cell lysis	3	6, 11 Feb				
	2. Filtration technique (such as ultrafiltration)/Dialysis and membrane filtration/Salting out (+Salting in)	1	21 (A) Jan	KW	2. Differential centrifugation 3. Salting out 4. Dialysis						
1.4 Enzyme (LDH) purification	Protein purification: 1. Chromatography (counter current, ion exchange, gel filtration, affinity, reverse phase) 2. Spectrophotometry	2	23, 28 (M) Jan 28 (A), 30 Jan	SS	1. Ion-exchange chromatography 2. Quantitative (Lowry's) and qualitative determination	4	13, 18, 20 Feb				
		2		MP		3	25, 27 Feb				
1.5 Enzyme activity assay and kinetics	1. Enzyme kinetics	1	4 (M) Feb	NW	1. Enzyme kinetics	2	11 (M) Mar				
	2. Protein gel electrophoresis	1	4 (A) Feb	PJ	2. Native and SDS PAGE	2	11 (A), 13, 18 (M) Mar				
Discussion							18 (A) Mar				

Mid-term examination: สอบ 3 - 7 Mar 2025; 04-03-2025 13:00 - 16:00 น.

2. Recombinant protein production								Pawinee	Pattana	Kittikhun	Karan
Overview of Recombinant protein production 2.1. Protein expression (carbohydrate modifying enzyme - Inulosucrase sucrase + His Taq)	1. DNA cloning 2. Recombinant protein production 3. Induction and control of gene expression	1	11 (M) Mar	KW	1. Reagent Preparatation 2. Protein expression (small scale to find the optimal condition, 100 ml, OD 0.6) 3. Induction of protein expression (vary IPTG concentration - 0, 0.05, 0.5 mM for 2 hours Crude protein preparation (centrifugation, detergent lysis to see whether protein is in soluble or insoluble fractions) - Boil the samples and store at -20 C	4	11 (A), 13, 18 Mar				1st
2.2. SDS-PAGE & Western blot	Western blot	1	20-Mar	PJ	4. Verification of protein expression by SDS-PAGE and WB - Gel preparation (4 gels/group) - SDS-PAGE to select optimal condition - WB using anti-His to verify the recombinant protein	2	25-Mar		1st		
2.3. Enzyme purification & buffer preparation	Affinity chromatography	1	27-Mar	KWB	5. Affinity chromatography - Students will be given a pellet - Lyse the cells by sonication method - Ni-column - SDS-PAGE + coomassie staining (Input, FT, W25, W50, E250) - pool fractions and dialysis	2	1-Apr				1st
2.4. Product determination (TLC)	TLC	1	3-Apr	PP	6. Enzymatic reaction (DNS assays by varying pH of the reacction buffers) 7. TLC (vary incubation times)	2	8-Apr	1st			
Discussion		1					10-Apr				

Final-term examination: สอบ 28 Apr - 14 May 2025; 06-05-2025 13:00 - 16:00 น.