CONCEPT: SPECIFIC ACTIVITY

●_____: a quantitative measure of the function & amount of target-protein.

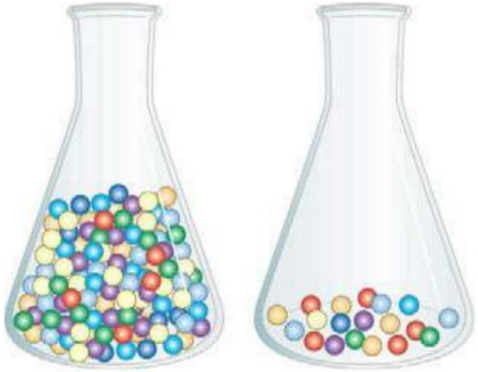
□ *Assay*: any test or _____ that measures the *presence* & *activity* of the target protein.

□ Assay is performed on a _____ portion of the protein sample to check for target-protein activity.

□ If two different samples have the same *activity*, then they have _____ amounts of target-protein.

EXAMPLE: Compare the *activity* of the red protein (●) in both flasks.

Flask #1 Flask #2



Which flask has greater activity?

a) Flask #1

b) Flask #2

c) Both flasks have equal activity.

Specific Activity Measures Protein Purity

●*Question*: how can biochemists tell if they have successfully purified a protein?

●Protein purity can be measured by the target-protein's _____ activity.

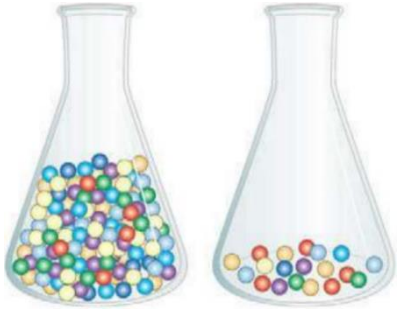
□ *Specific activity*: _____ of target-protein activity over the *total* amount of *all* protein present.

□ Specific activity _____ during purification & becomes *maximal/constant* with a pure protein.

EXAMPLE: Compare the *specific activity* of the red protein (●) in both flasks.

$$\text{Specific Activity} = \frac{\text{Activity}}{\text{Total Amount of Protein}}$$

Flask #1 Flask #2



Which flask has greater specific activity?

a) Flask #1 b) Flask #2

c) Both flasks have equal specific activity.

Purification Technique/Step	Volume of Sample (mL)	Total Protein (mg)	Target-Protein Activity (units)	Specific Activity (units/mg)
1. Crude Cellular Extract	1,400	10,000	100,000	10
2. Salting Out	280	3000	96,000	32
3. Ion-Exchange Chromatography	90	400	80,000	200
4. Size-Exclusion Chromatography	80	100	60,000	600
5. Affinity Chromatography	6	3	45,000	15,000
6. 2nd Affinity Chromatography	4	2	30,000	15,000

*_____ specific activity means a more purified protein.

CONCEPT: SPECIFIC ACTIVITY

PRACTICE: A biochemist discovers and purifies a new enzyme and generates the purification table below.

A) Fill-in the table below with the specific activity of the enzyme after each purification procedure.

Purification Technique/Step	Volume of Sample (mL)	Total Protein (mg)	Target-Protein Activity (units)	Specific Activity (units/mg)	Relative Purity
1. Crude Cellular Extract	1200	20,000	4,000,000	_____	N/A
2. Differential Centrifugation	350	12,000	3,000,000	_____	_____
3. Salting Out	110	4,000	1,500,000	_____	_____
4. Ion-Exchange Chromatography	60	200	800,000	_____	_____
5. Size-Exclusion Chromatography	35	50	750,000	_____	_____
6. Affinity Chromatography	5	45	675,000	_____	_____

B) According to the data, which purification step was most effective to give the greatest *relative* increase in purity?

_____

$$\text{Relative Purity of Technique} = \frac{\text{(Specific Activity of Technique)}}{\text{(Specific Activity Before Technique)}}$$

C) Which purification step was least effective to give the lowest relative increase in purity?

_____

D) Based on the results in the table, should the biochemist be convinced that the enzyme is pure after step 6? Why?