OPTIMIZATION OF ENZYME COMPOSITIONS USING DE COLLAGENASE PRODUCTS

Key Assumptions:

- Collagenase & protease activity are required to release cells from tissue or recover cells after culture on a collagen matrix.
- DE Collagenase contains highly enriched collagenase with minimal protease activity.
- Highly enriched or purified collagenase has restricted specificity: degrades native or denatured collagen (i.e., gelatin).
- Excess collagenase will not damage cells because of the restricted specificity and inability to damage or degrade other proteins.
- Control of neutral protease activity in collagenase-protease mixtures are critical to maximize recovery of viable, functional cells.

TITRATION OF NEUTRAL PROTEASE ACTIVITY

Step 1: Set a starting point based on mass of collagenase used in your isolation procedure. Illustration below:

- For example, if 1 mg/mL of Worthington Biochemicals Corporation Type 1 collagenase (WBC T1) has always worked in your isolation procedure; you can safely assume collagenase is in excess at this dose.
- Set 1 mg/mL of WBC T1 collagenase as the reference point to keep collagenase in excess as you titrate neutral protease activity (NPA).

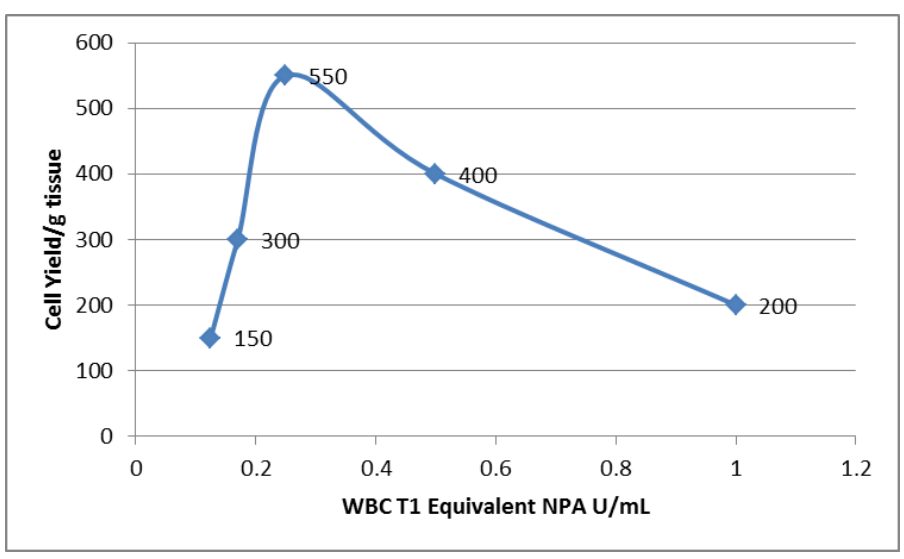
Step 2: Titer the amount of NPA required for your cell isolation procedure keeping DE Collagenase 10/100 at 1 mg/mL.

- DE Collagenase 10/100 has the same collagenase and NPA as WBC T1collagenase.
- To perform titration, adjust the mass of the DE Collagenase product so the mass of collagenase is equivalent to that found in 1 mg/mL of WBC T1 collagenase as illustrated below:

	DE 10/100	DE 20/200	DE 40/400	DE 60/600	DE 80/800
mg/mL DE Collagenase	1 mg/ml	0.5 mg/mL	0.25 mg/mL	0.17 mg/mL	0.125 mg/mL
WBC T1 Collagenase equivalence	≈ WBC T1	≈ WBC T1	≈ WBC T1	≈ WBC T1	≈ WBC T1
WBC T1 NPA equivalence	≈ WBC T1	≈ 50% WBC T1	≈ 25% WBC T1	≈ 17% WBC T1	≈ 12% WBC T1

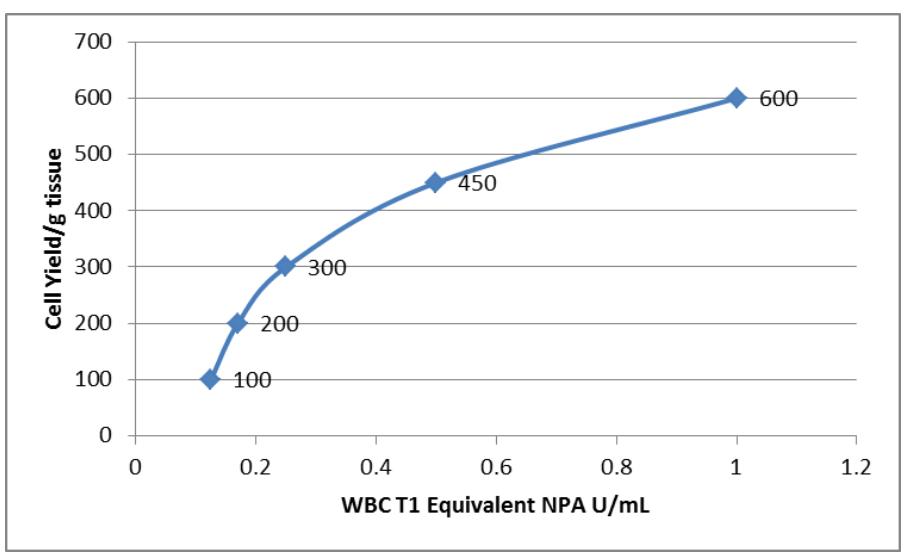
FOUR POSSIBLE RESULTS

Optimal Enzyme Formulation



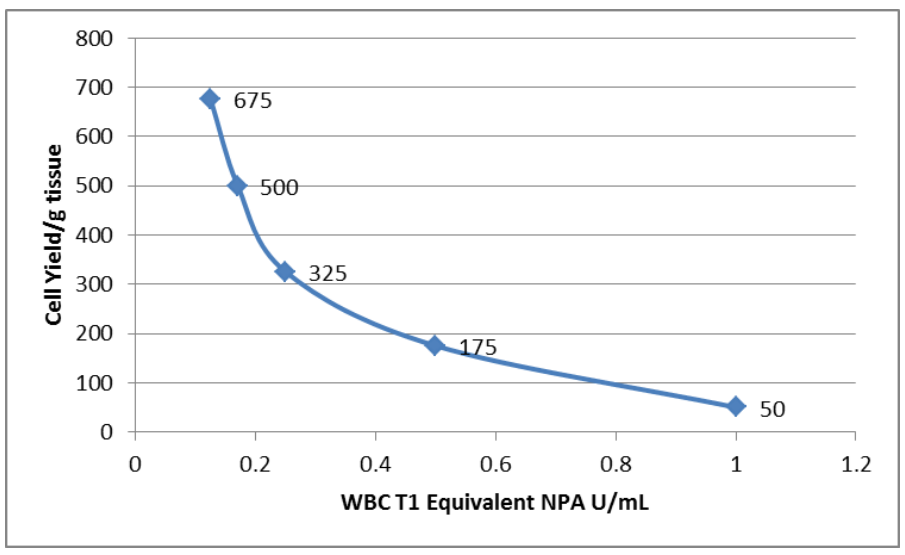
Non-Optimal Enzyme Formulation

Upper Limit Neutral Protease Activity Not Defined



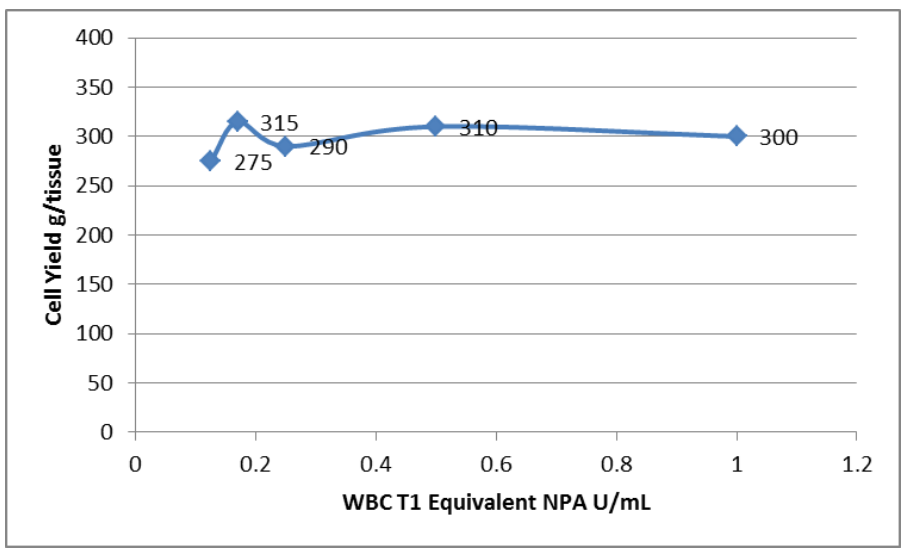
Non-Optimal Enzyme Formulation

Lower Limit Neutral Protease Activity Not Defined



Optimal Enzyme Formulation

Neutral Protease Activity Has No Effect On Outcome



Conclusion: The results from the hypothetical experiment match those in upper left hand plot, optimal amount of neutral protease activity is equivalent to 0.25 mg/mL of WBC T1.

TITRATION OF COLLAGENASE AGAINST FIXED NEUTRAL PROTEASE ACTIVITY

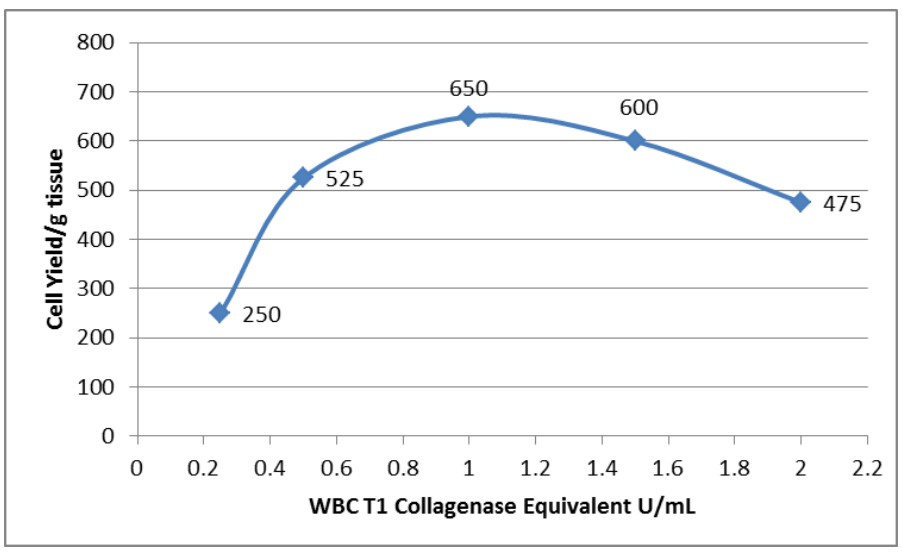
Step 3: Set the neutral protease reference point as equivalent to 0.25 mg/mL of WBC T1. Illustration below:

- All of the DE Collagenase products contain the same amount of neutral protease per mg of product so use DE Collagenases at 0.25 mg/mL.

	DE 10/100	DE 20/200	DE 40/400	DE 60/600	DE 80/800
mg/mL DE Collagenase	0.25 mg/ml	0.25 mg/mL	0.25 mg/mL	0.25 mg/mL	0.25 mg/mL
WBC T1 equivalent NPA	≈ 0.25 mg/mL WBC T1	≈ 0.25 mg/mL WBC T1	≈ 0.25 mg/mL WBC T1	≈ 0.25 mg/mL WBC T1	≈ 0.25 mg/mL WBC T1
WBC T1 equivalent collagenase	≈ 25% WBC T1	≈ 50% WBC T1	≈ 100 % WBC T1	≈ 150 % WBC T1	≈ 200 % WBC T1

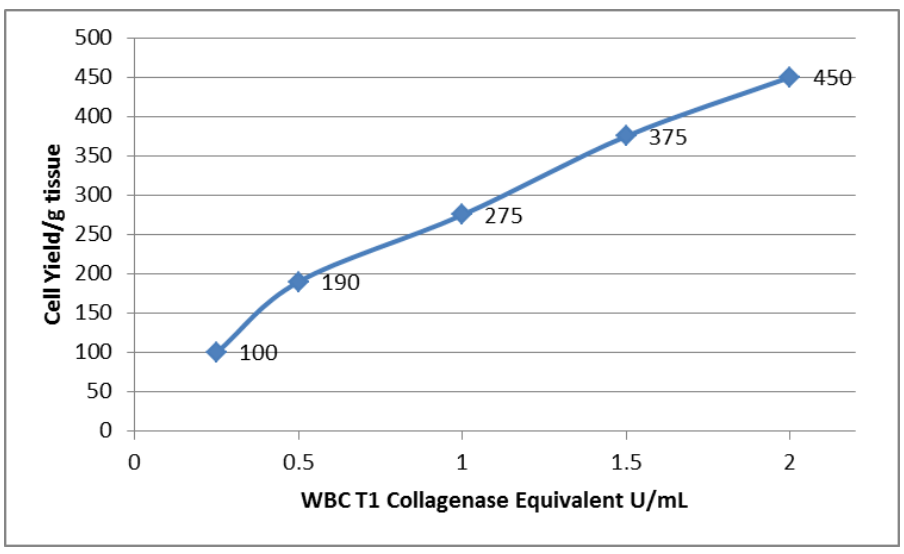
FOUR POSSIBLE RESULTS

Optimal Enzyme Formulation



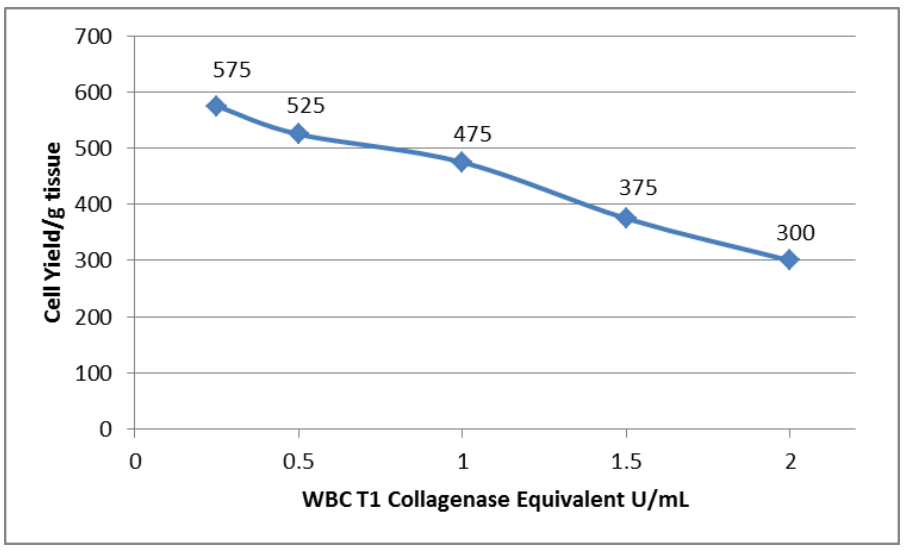
Non-Optimal Enzyme Formulation

Upper Limit Collagenase Activity Not Defined



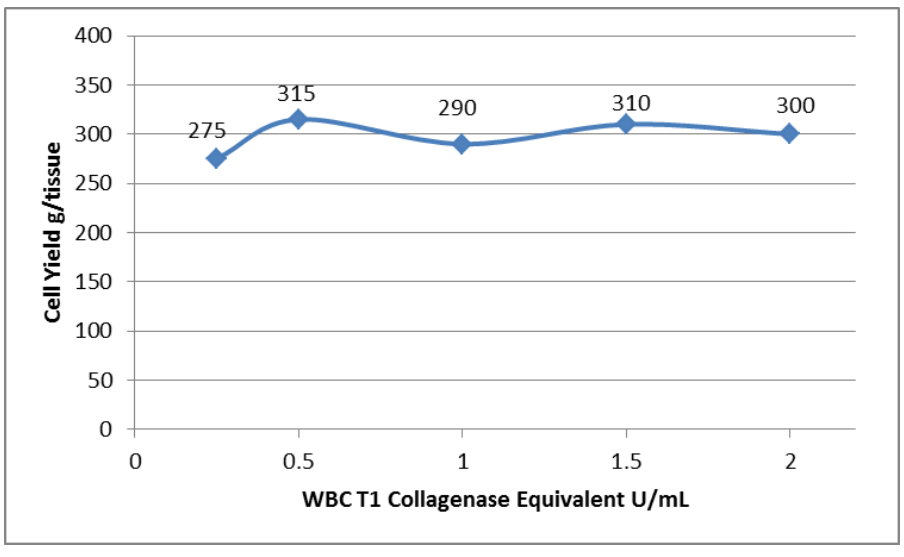
Non-Optimal Enzyme Formulation

Lower Limit Collagenase Activity Not Defined



Optimal Enzyme Formulation

Collagenase Activity Has No Effect On Outcome



Conclusion:

- Results from titration from this hypothetical experiment match those from the upper left hand plot
- Conclude that optimal composition collagenase to isolate the specific cell population is 0.25 mg/mL DE Collagenase 40/400 that is equivalent to collagenase found in 1 mg of WBC T1 Collagenase.

Step 4: Optional step: verify that 0.25 mg/mL of DE Collagenase 40/400 is the optimal dose of enzyme required for this application by titrating the dose of DE Collagenase 40/400 used in the isolation procedure.

References:

- Blog post: "Learning from lot qualification, RC McCarthy , 7 December 2017: https://www.vitacyte.com/news/lot-qualification/.
- White Paper: "DE Collagenase Optimization Kit: a fresh approach to defining enzyme composition and dose for maximal cell recovery: https://www.vitacyte.com/vitacytes-publications-presentations/de-collagenase-optimization-kit/.