

## Day One

Prepare CFAI media and sugar solution for overnight culture (autoclave)  
**2 hrs**

Inoculate CFAI media and sugar solution, grow overnight  
**10 min, wait 14 hrs**

### A. CFAI

## Day Two

Pellet, wash, and freeze cells  
**1.5 hrs**

Prepare extract  
**2.5 hrs**

Set up CFPS batch reactions  
**10 min per 4 rxns, wait 4 hrs**

Quantification of protein production  
**5 min per 4 rxns**

## Day One

Prepare LB for overnight culture (autoclave)  
**2 hrs**

Inoculate 50 mL LB overnight culture from plate  
**10 min, wait 20 hrs**

### B. Standard Method

## Day Two

Prepare 2x YPT and Glucose for 1L growth (autoclave)  
**2.5 hrs**

Inoculate and grow 1L to final OD of approximately 3.0  
**3 hrs**

Pellet, wash, and freeze cells  
**1.5 hrs**

Prepare extract  
**2.5 hrs**

## Day Three

Set up CFPS batch reactions  
**10 min per 4 rxns, wait 4 hrs**

Quantification of protein production  
**5 min per 4 rxns**