S30 cell free kit protocol - 1 reaction

* For more than one reaction, multiply all amounts by the number of reactions required (i.e. if 2 reactions required, MM would contain 10µl amino acid mix, 40µl S30 premix, and 30µl S30 extract, circular)
*Remember to account for the cell free kit control when determining the number of reactions required
*Typical protein yield is between 50 and 250 ng per 50µl reaction (i.e. 1 - 5ng/µl of protein)

Master mix preparation:

*Keep on ice
*Make up an extra 0.25 of a reaction (i.e. 1.25µl AA (0.63+0.63), 5µl S30 premix, 3.75µl S30 extract) to ensure accurate pipetting

a. Prepare 5µl of complete amino acid mix by mixing together two incomplete amino acid mixes (e.g. minus cysteine and minus leucine) in equal amounts, i.e. 2.5µl + 2.5µl
b. Prepare the cell free kit master mix in this order:
    - S30 premix (gently mixed): 20µl
    - S30 extract, circular (gently mixed): 15µl
    - Amino acid mix: 5µl (from previous step)

Reaction set-up:

*Prepare on ice
*Set up a separate reaction for each plasmid to be expressed

- Master mix: 40µl
- Nuclease-free water: up to 50µl
- DNA template: ≤ 2µg

*Also set up a cell-free control

- pBEST*luc*™ DNA (1µg/µl): 2µl
- Master mix: 40µl
- Nuclease-free water: up to 50µl

Procedure:

1. Vortex gently and centrifuge for 5 seconds
2. Incubate reactions at 37C for 60 minutes
3. Stop reaction by placing on ice for 5 mins

Luc control measurement:

1. Prepare four 2 in 1 serial dilutions of the Luc cell free reaction by mixing 50µl of luciferase dilution reagent and 50µl of cell free reaction at RT
2. Add 50µl of luciferase reagent to each dilution and measure luminescence immediately