

Warm-up exercise

What are the ranges of the pipettes at your table?

- 1.
- 2.
- 3.
- 4.

Select the correct pipette for desired volume:

- | | | | |
|------------|-------|------------|-------|
| 1. 1.25 ul | _____ | 5. 9.8 ul | _____ |
| 2. 165 ul | _____ | 6. 2.00 ul | _____ |
| 3. 200ul | _____ | 7. 12.8 ul | _____ |
| 4. 20 ul | _____ | 8. 465 ul | _____ |

Exercise 1 & 2.

Each student should receive four 1.5 ml tubes. Before pipetting label your tubes A,B,C,D.

Exercise 1. Adding & Removing large volumes

1. Add 370 *ul* Blue reagent to tube A.
2. Add 225 *ul* of Red reagent to tube C.
3. Add 495 *ul* of Yellow Reagent to tube B.
4. Add 160 *ul* of Blue reagent to tube D.
5. Remove 155 *ul* from tube A and dispose.
6. Remove 190 *ul* from tube B and add it to tube D (do not mix).

Exercise 2. Adding vol. into vol. & common mixing techniques. (Note before and after mixing).

1. Add 285 μ l of Red reagent to tube A and mix by pipetting up and down.
2. Add 195 μ l of Blue reagent to B and mix using inversion.
3. Add 275 μ l of Yellow reagent to tube C and mix using flick/tap method.
4. Add 150 μ l of Red reagent to tube D and mix by vortexing.

Exercise 3 & 4.

Each student should receive four 200ul tubes & label them E,F,G,H.

Exercise 3. Adding small volumes

1. Add 20 μ l of Blue reagent to tube E.
2. Add 11 ul of Red reagent to tube F.
3. Add 2.5 ul of Yellow reagent to tube G.
4. Add 1 ul of Blue reagent to tube H.

Exercise 4. Removing & mixing small volumes

1. Remove 10 ul from tube E and dispose of it.
2. Remove 1 ul from tube F and dispose of it.
3. Add 7.5 ul of Red reagent to tube G and mix by pipetting up and down.
4. Add 9 ul of Yellow reagent to tube H and mix by pipetting up and down.

Exercise 5. Making a master mix. (1-1.5 ml tube per person) Often to save on time and to reduce variation between reactions, it is helpful to make a mastermix when doing multiple reactions. Mastermixes are made by making reaction mixes that are larger in total volume but contain the same ratios of reactants as 1x. It is often customary to make mastermixes larger than what is desired to account for pipetting error. Example Desired =5x , Make = 5.5x Desired =20x make=22x

1x Reaction	Desired 9x	Make ___x Reaction
10 ul Yellow reactant.	___Yellow Reactant.	___Yellow Reactant
9ul Blue reactant	___Blue Reactant	___Blue Reactant
1ul Red reactant	___Red Reactant	___Red Reactant
20ul Total	___Total	___Total