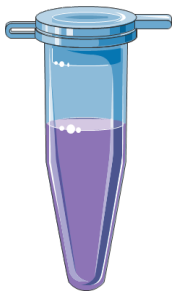
**3.1. Calculate the seeding volume needed, $V_S$**

e.g. for 2 gel precursors:
$V_S$ = 1.1 x (2 x 250 µL)
$V_S$ = 550 µL



**3.2. Calculate the required volume of each matrix component**

e.g. from a 3 mg/mL collagen stock, we want 100 µg/mL in the final gel.

Volume of collagen stock $V_C$:
$V_C = (V_S \times 100 \times 5)/3000$
$V_C$ = 92 µL

**3.3. Store each required matrix component on ice**

**3.4. If adding acidic collagen, make up neutralisation volume, $V_N$**

Typically, we use $V_N = 2 \times V_C$ so here $V_N$ = 184 µL

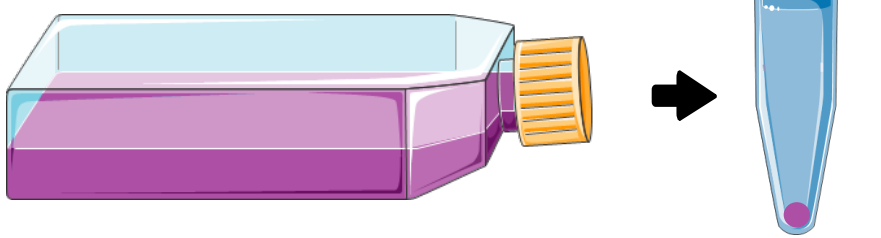
Mix NaOH and 10X PBS:
$V_{NaOH} = 0.023 \times V_C$ = 2.1 µL
$V_{PBS} = (V_C + V_N)/10$ = 27.6 µL

Make up to $V_N$ with sterile $H_2O$.
DO NOT ADD COLLAGEN AT THIS POINT

**Step 4**
**Prepare the cell pellet needed for seeding**

e.g. for $1x10^5$ cells/mL in final gel, in $V_S$ we need $5x10^5$ cells/mL (due to 1:5 dilution)

Cells required = $5x10^5 \times V_S$
Since $V_S$ = 550 µL
Cells required = $2.75x10^5$



**Step 5**
**Mix all components, make up to $V_S$ using cell culture medium, add to precursors, and seed**