Cell Lysis Protocol

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| --- | --- | --- |
| Reagents for Lysis Buffer | | |
|  | 1x (µL) | 0 |
| Tris (1M) | 0.15 | 0.00 |
| EDTA (100µM) | 0.10 | 0.00 |
| Potassium chloride (100µM) | 1.00 | 0.00 |
| Triton (5%) | 0.20 | 0.00 |
| DTT (1M) | 0.20 | 0.00 |
| Protease/ Proteinase K (20mg/mL) | 0.125 | 0.000 |
| Ultrapure water | 3.225 | 0.000 |
| Total | 5.000 | 0.000 |

Note: To update table, change the ‘0’ in the last column of the second row to the number of buffers needed (only enter a numeric value). Then, highlight the entire third column and press ‘F9’.

Creation and Distribution of the Master Mix

1. Determine how much buffer is required. Always make a bit more than necessary.

Ex. If you are preparing buffer for 8 samples, make 9x or 10x of buffer.

1. Using the table above, create a master mix in an eppendorf tube with the amounts specified in the last column. **DO NOT** make buffer for each sample separately. Prepare a single large amount of solution that will be enough for all the samples.
2. For each sample you intend to lyse, transfer 5µL of the buffer solution to a well on a 96 well plate.

Note: There should be a small amount of solution left over when you are done transferring

Thermal Cycler Program

1. After samples have been added to cell lysis buffer (whether by pipette or cell sorting machine), firmly seal the plate with a cover to prevent evaporation of sample during thermal cycle process.
2. Preheat the machine with a **50°C Hold**, before adding the plate to the machine.
3. Once the lid reaches 50°C, add the plate and run the next rest of the program:
4. **50°C for 3 hours** (optimal temperature to enhance protease activity)
5. **75°C for 20 minutes**
6. **80°C for 5 minutes** (denatures protease)
7. **4°C Hold** (this step preserves the DNA sample until it can be retrieved)
8. After sample reaches 4°C, you may safely store the samples in a 4°C refrigerator for a month or two.