Reagents needed:

Swab Collection

1. FLOQSwabs
2. FBS
3. DMSO
4. Mr Frosty Freezing Container
5. Dry ice
6. Dry ice container
7. Cryovials

Single cell preparation

1. FBS
2. DMSO
3. RPMI
4. Accutase
5. DTT
6. Trypan Blue
7. NI haemocytometer
8. Beta-mercaptoethannol
9. RLT buffer
10. Cell strainer
11. Thermomixer
12. Cryovials

Sample collection protocol (from Zeigler et al Cell 2021)

1. Nasopharyngeal samples were collected by a trained healthcare provider using FLOQSwabs (Copan flocked swabs) following the manufacturer’s instructions.
2. The patient’s head was tilted back slightly, and the swab inserted along the nasal septum, above the floor of the nasal passage to the nasopharynx until slight resistance was felt (Video available)
3. The swab was then left in place for several seconds to absorb secretions and slowly removed while rotating swab.
4. The swab was then placed into a cryogenic vial with 900 μL of heat inactivated [fetal bovine serum](https://www.sciencedirect.com/topics/neuroscience/fetal-bovine-serum) (FBS) and 100 μL of [dimethyl sulfoxide](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/dimethyl-sulfoxide) (DMSO).
5. Vials were placed into a Mr. Frosty Freezing Container (Thermo Fisher Scientific) for optimal cell preservation. A Mr. Frosty containing the vials was placed in a cooler with dry ice for transportation from patient areas to the laboratory for processing.
6. Once in the laboratory, the Mr. Frosty was placed into a −80°C freezer overnight, and on the next day, the vials were moved to liquid nitrogen storage containers.

Single cell preparation (from Zeigler et al Cell 2021)

1. Swabs in freezing media (90% FBS/10% DMSO) were stored in liquid nitrogen until immediately prior to dissociation.
2. A detailed sample protocol can be found here: <https://protocols.io/view/human-nasopharyngeal-swab-processing-for-viable-si-bjhkkj4w.html>.
3. Nasal swabs in freezing media were thawed, and each swab was rinsed in RPMI before incubation in 1 mL RPMI/10 mM DTT (Sigma) for 15 min at 37°C with agitation.
4. Next, the nasal swab was incubated in 1 mL Accutase (Sigma) for 30 min at 37°C with agitation.
5. The 1 mL RPMI/10 mM DTT from the nasal swab incubation was centrifuged at 400 g for 5 min at 4°C to pellet cells, the supernatant was discarded, and the cell pellet was resuspended in 1 mL Accutase and incubated for 30 min at 37°C with agitation.
6. The original cryovial containing the freezing media and the original swab washings were combined and centrifuged at 400 g for 5 min at 4°C.
7. The cell pellet was then resuspended in RPMI/10 mM DTT, and incubated for 15 min at 37°C with agitation, centrifuged as above, the supernatant was aspirated, and the cell pellet was resuspended in 1 mL Accutase, and incubated for 30 min at 37°C with agitation.
8. All cells were combined following Accutase digestion and filtered using a 70 μm nylon strainer.
9. The filter and swab were washed with RPMI/10% FBS/4 mM EDTA, and all washings combined.
10. Dissociated, filtered cells were centrifuged at 400 g for 10 min at 4°C, and resuspended in 200 μL RPMI/10% FBS for counting.
11. Cells were diluted to 20,000 cells in 200 μL for scRNA-seq.