

Freezing human cell lines

35 mm \rightarrow 0.1 ml
60 mm \rightarrow 0.5 ml
10 cm \rightarrow 1 ml
15 cm \rightarrow 2.5 ml

1: $\frac{5}{6}$
or

10 cm plate.

1 ml Trypsin 2-5 min detach.

5 ml CM

cell counting. 10 ml cells premix
+
10 ml dye (Trypan Blue stain 0.4%)

cryovial tube (2 ml). + print label.

centrifuge 5 min. 9000g

2 ml HEK
< 3 ml HT115
discard/remove medium

+ equal volume
Freezing medium

50% EBS	25 ml
40% DMEM	20 ml
10% DMSO	5 ml

-80°C for 1 day

liquid nitrogen for long term storage

others

freezing bacteria. easy thaw.

freezing lentivirus easy just filter it

L-WRN cell line

HT29 ^{or} hard detach
10-20 min

2 ml PBS 1 min
wash medium
PBS

\rightarrow wall.

HEK: 1.21×10^6
 1.11×10^6 ✓

HT115: 6.89×10^5
 4.81×10^5 ✓

after

HEK: 2.19×10^6
 7.32×10^5 ?

HT115: 1.78×10^5
 1.57×10^5