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□□ □□	37°C, 5%CO2, □□ □□.
□□ □□	Subculture reagents as recommended on the certificate of analysis

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Subculture the cells at about 75 % confluence. Denser cultures undergo irreversible contact inhibition and stop proliferating. Use the subculture reagents recommended on the certificate of analysis.

□□ □□	More than 6,000 cells/cm²
□□ □□	24 hours after seeding, then every 48 to 72 hours
□□ □ □□	Post-thaw viability ≥ 70 % viable cells. More than 80 % confluence within the expected recovery period.

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Thaw the cryovial quickly in a 37 °C water bath, swirling gently. Just before the last ice has melted, move the vial to a laminar flow hood and decontaminate its outer surface.
Transfer the cell suspension into a 15 ml centrifuge tube, add 10 ml of keratinocyte growth medium and centrifuge at 250 × g for 5 minutes.
Remove the supernatant and resuspend the cell pellet in 10 ml of keratinocyte growth medium. Check cell number and viability.
Divide the suspension between two 25 cm² culture flasks (5 ml each). Using two flasks reduces the risk of losing all cells if a passage fails.
Incubate at 37 °C, 5 % CO_2 and saturated humidity, with the screw caps opened by half a turn for gas exchange.
Change the medium after 24 hours to remove unattached cells, then every 48 to 72 hours, using pre-warmed medium only.
Subculture the cells at about 75 % confluence. Denser cultures undergo irreversible contact inhibition and stop proliferating. Use the subculture reagents recommended on the certificate of analysis.
Recommended seeding density: more than 6,000 cells/cm².

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