

| Problem                   | Possible Reasons                                                                            | What to do?                                                                                                                      |
|---------------------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Change of cell morphology | Cross-contamination with other cultured cells/cell lines                                    | Check sterile working technique; only work with one cell line/cell type at a time; use separate media bottles for each cell type |
|                           | Unspecific isolation protocol                                                               |                                                                                                                                  |
|                           | Shift from serum-containing to serum-free medium or other modification of media composition | Check with medium used before                                                                                                    |
|                           | Mycoplasma contamination                                                                    | Detect and eliminate; use sterile techniques according to the guidelines for good cell culture practice                          |
|                           | Cells have become senescent or have terminally differentiated                               | Freshly isolate or thaw cells from stock; check your culture conditions                                                          |

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