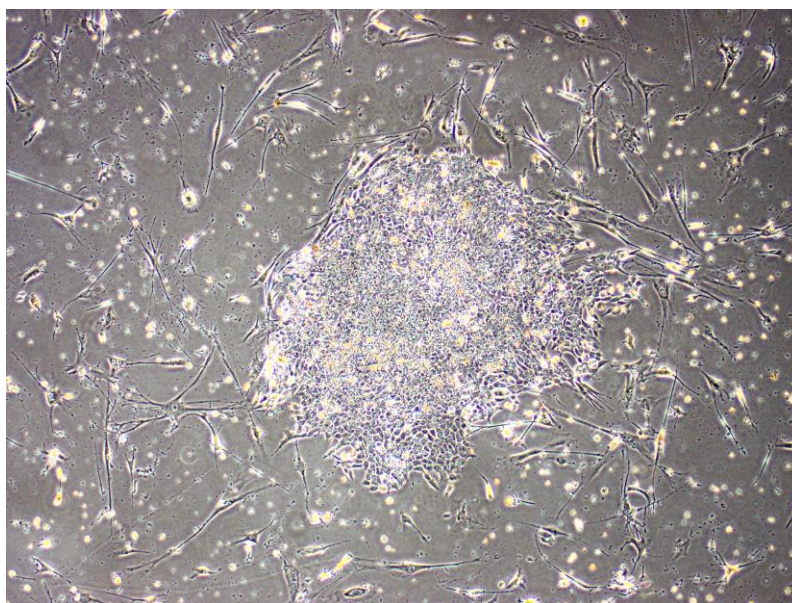


## Application consideration

### AES0174 : rdES2-1

Please keep in mind the following points:

- Please make sure to count the number of cells after thawing as well as during subculturing. And then start cultivating the cells by diluting them to the recommended cell density ( $0.5-1.0 \times 10^4$  cells / 60 mm dish or T25 flask).
- Please subculture the cells after you see a colony as one shown in the morphological photo below (Since the cell proliferation declines right after thawing, 4 to 5 days are required for subculturing cells).
- For washing the cells during subculturing, please use DMEM/F12 medium that does not contain KnockOut Serum Replacement (KSR).
- For detaching the cells during subculturing, please use 0.1% trypsin diluted with DMEM/F12 medium that does not contain KSR.
- For subculturing cells, they should be dispersed into single cells.



Colony of cells with indistinct boundaries (Rabbit ES cells : 4-5 days after thawing).

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