2025-11

Application consideration

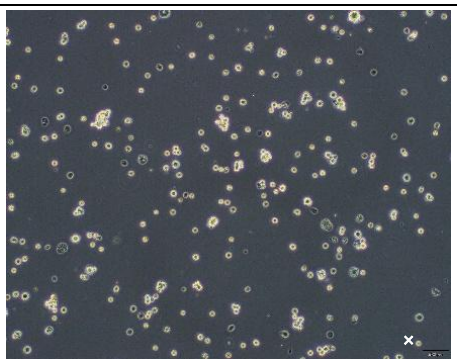
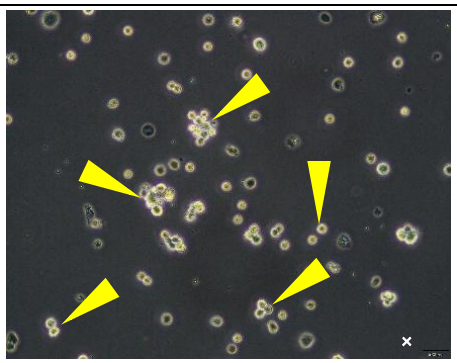
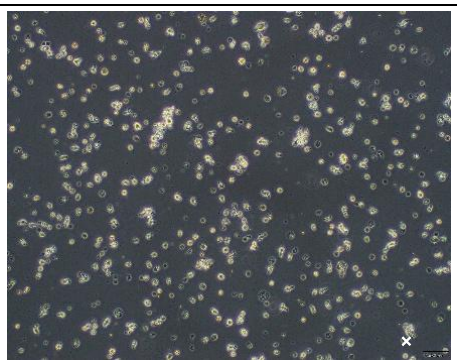
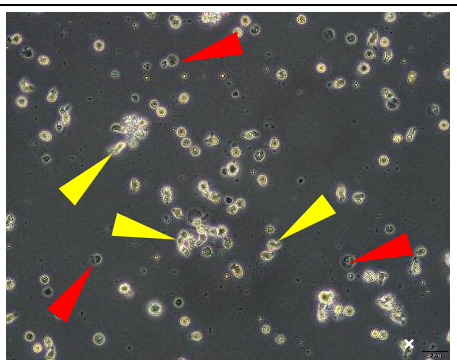
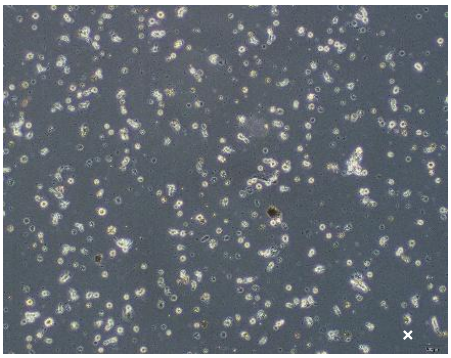
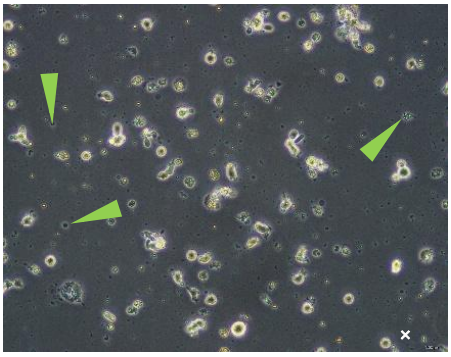
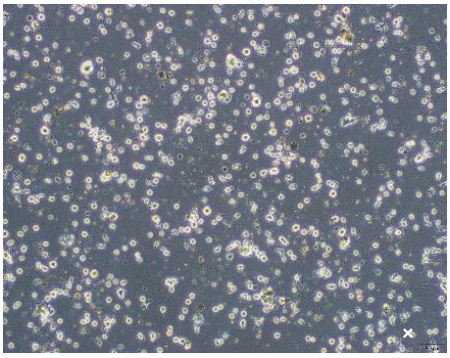
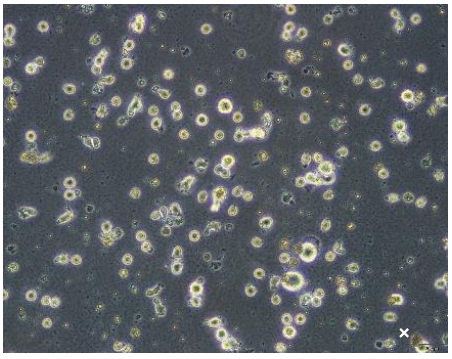
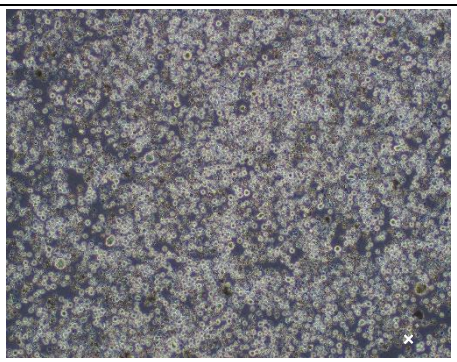
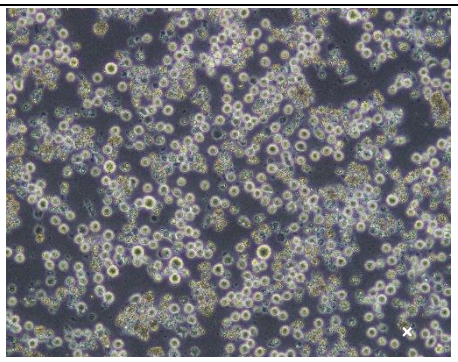
RCB5684 : MDS92

Please keep in mind the following points :

- **These cells proliferate very slowly, making stable culture difficult. Although gradual proliferation occurs, many cells undergo differentiation and subsequently die, resulting in approximately half of the cell population being non-viable. Consequently, a large amount of cellular debris is often observed, which may resemble contamination.**
- **The cells are highly - susceptible to damage during freezing and thawing. Therefore, thaw one vial of cells at a time, and complete all handling as quickly as possible. Immediately transfer the cells to an incubator after thawing.**
- **Many cells die during the freeze-thaw process. Suspend the cells in 4 mL of medium and seed 2 mL into each of two T12.5 cm² flasks. Due to slow proliferation and medium evaporation during culture, please use vented-cap flasks or plug-sealed flasks with loosened caps. Dishes and plates are unsuitable because they increase the risk of medium evaporation during cultivation.**
- **Add IL-3 every 3 to 4 days to reach a final concentration of 50 ng/mL. Do not take into account any residual IL-3 already present in the medium.**
- **One to two weeks after thawing, add 0.2 to 0.5 mL of medium and an amount of IL-3 sufficient to reach a final concentration of 50 ng/mL per T12.5 flask.**
- **It takes approximately 3 to 4 weeks for the cells to begin proliferating reliably. During this period, avoid unnecessary handling. Limit culture operations to the addition of cytokines or small amounts of medium (add once every 1 to 2 weeks). Minimize any stress to the cells during this period.**
- **Even after stable cell proliferation is established, the cells grow slowly and can be passaged only once every 1 to 2 weeks (at a split ratio approximately of 1:1.5 to 2.0). When the cell density reaches around 2.0 x10^5 cells/mL, dilute them to approximately 1.0 to 1.5 x 10^5 cells/mL.**
- **If possible, perform Giemsa staining regularly to monitor cell morphology, including the presence of cells at various stages of maturation, apoptotic cells, and occasional mitotic figures. During prolonged culture, if proliferating cells become scarce or, conversely, if the growth rate increases abnormally, immature cells may dominate the population, potentially resulting in the loss of the original characteristics of MDS cell line. Please refer to the images provided in our online catalog for further details.**

[See the reverse side for images showing the process from thawing to passaging.]

【Technical support】e-mail : cellqa.brc@riken.jp

		**Immediately after thawing:** Cell density is low, but bright, round viable cells are visible. (Yellow)
		**One day after thawing:** Approximately half of the cells are dead (Red), and the surviving cells exhibit slight morphological abnormalities. (Yellow)
		**Five days after thawing:** No signs of proliferation have been observed since the day after thawing. Small dark dots, likely cell debris, are visible but not indicate contamination. (Green)
		**Eighteen days after thawing:** Following the addition of IL-3 every 3–4 days and a small amount of medium with IL-3 once a week, the cells have begun to proliferate gradually. No passaging has been performed to date.
		**Timing for Passaging:** When the cell density reaches approximately 2 x10^5 cells/mL, dilute the culture to 1 to 1.5 x10^5 cells/mL. Centrifugation to remove dead cells is not necessary.

*Images on our website can be enlarged for detailed viewing.

Regular Giemsa staining is recommended to monitor cell status.

< Cytospin May-Giemsa Staining of MDS Cells >

(Provided by the depositor)

A photo of cytospin preparation with May-Giemsa staining.

Most of the cells gradually and spontaneously differentiate into dysplastic neutrophil-like or macrophage-like or apoptotic cells but a small part of the cells retain immature blastic features.