**Troubleshooting of tumor organoid culture establishment**

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| **Reagent/consumable** | **Benefits** |
| Rho kinase inhibitor (ROCK) | Avoid cell apoptosis and enhance cell-cell adhesion, enhance cell viability of the organoid culture, particularly after freezing |
| Heparin | Enhance the expansion of stem cells and cell proliferation and cell viability of the organoid culture |
| Accutase | Gentle digestion reagent to detach cells that maintains cell viability better than using other reagents, such as Trypsin |
| DNase | Eliminate DNA remnants from dead and necrotic cells and prevents clumping of cells during tissue processing |
| Red blood cell lysis reagent | Remove red blood cell contamination of the tissue sample |
| Specialized digestion media and/or mechanical dissociation equipment | Aid in the dissociation of hard tumor tissues with a high stroma content to obtain isolated single tumor cells |
| Ultra-low attachment (ULA) plates | Reduces the growth of adherent fibroblasts |