Soft Agar Colony Formation Assay

Anchorage-independent growth assay for transformation and tumorigenicity

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Overview

The soft agar colony formation assay is a gold standard method for measuring anchorage-independent growth, a hallmark of cellular transformation and tumorigenicity. Normal cells require attachment to a solid substrate for growth, while transformed cells can proliferate in semi-solid medium.

Principle:

Cells are suspended in low-concentration agar (0.3-0.6%) layered over a higher-concentration base agar (0.5-1.0%). Transformed cells form colonies in suspension, while normal cells cannot proliferate without attachment. Colony formation is quantified after 2-4 weeks.

Applications

- Assessing tumorigenic potential
- Testing tumor suppressor function
- Oncogene transformation studies
- Drug screening for cancer cells
- Evaluating metastatic potential
- Quality control for cell lines

Advantages

- Highly predictive of in vivo tumorigenicity
- Quantitative and reproducible
- Can assess thousands of cells per well

- Does not require animal models
- Suitable for high-throughput screening

Expected Timeline:

- **Day 0:** Prepare agar layers and seed cells
- **Day 1-3:** Monitor for contamination and agar integrity
- **Week 2-3:** Colonies become visible (>50 μm)
- **Week 3-4:** Count and analyze colonies
- **Total duration:** 21-28 days

Important Considerations

- Agar must remain molten (42°C) but not so hot it kills cells
- Work quickly to prevent agar from solidifying prematurely
- Maintain sterile technique throughout - assay lasts 3-4 weeks
- Use low-passage cells for reproducible results
- Include positive and negative controls