Certified Specialist Programme in Cell Culture

Primary Cell Isolation and Maintenance

Aseptic Technique
Concept: Procedures to prevent microbial contamination during cell handling.
Related terms: sterile field, biosafety cabinet, disinfectant
Explanation: Involves using sterilized instruments, wearing gloves, and working within a laminar flow hood to maintain a contaminant-free environment.
Example: Disinfecting the work surface with 70% ethanol before opening a tissue specimen.
Application: Essential for isolating viable primary cells from animal or human tissues.
Challenge: Human error such as touching non-sterile surfaces can introduce microbes, compromising cultures.

Antibiotics
Concept: Chemical agents added to culture media to suppress bacterial growth.
Related terms: penicillin-streptomycin, gentamicin, antimycotics
Explanation: Commonly used at low concentrations; they do not replace proper aseptic technique but provide a safety net.
Example: Adding 100 U/mL penicillin and 100 µg/mL streptomycin to a fibroblast growth medium.
Application: Helpful when tissue samples have high bacterial load.
Challenge: Overreliance can mask low-level contamination and may affect cell physiology.

Apoptosis Assay
Concept: Method to assess programmed cell death in isolated primary cells.
Related terms: Annexin V, caspase activity, flow cytometry
Explanation: Detects phosphatidylserine externalization or caspase activation, indicating cell health post-isolation.
Example: Staining freshly isolated hepatocytes with Annexin V-FITC and propidium iodide.
Application: Determines the impact of enzymatic digestion on cell viability.
Challenge: Rapid loss of viability can be misinterpreted if timing is not standardized.

Basal Medium
Concept: Core nutrient solution supporting basic cellular metabolism.
Related terms: DMEM, RPMI-1640, MEM
Explanation: Provides amino acids, vitamins, salts, and glucose; often supplemented with serum or growth factors.
Example: Using Dulbecco's Modified Eagle Medium as the base for neuronal cultures.
Application: Forms the foundation for custom media formulations.

Challenge: Inadequate buffering or nutrient levels can lead to poor cell growth.

Biopsy
Concept: Small tissue sample obtained for diagnostic or research purposes.
Related terms: needle biopsy, core biopsy, surgical excision
Explanation: Provides the starting material for primary cell isolation; must be processed quickly to preserve cell viability.
Example: Obtaining a liver core biopsy for hepatocyte isolation.
Application: Enables patient-specific cell studies and personalized medicine.
Challenge: Limited tissue size restricts cell yield; risk of contamination is higher if not handled aseptically.

Collagenase
Concept: Enzyme that degrades collagen to release cells from extracellular matrix.
Related terms: enzyme cocktail, tissue dissociation, trypsin
Explanation: Used at specific concentrations and temperatures to gently dissociate tissues while preserving surface receptors.
Example: Incubating pancreatic tissue in 0.5 mg/mL collagenase for 30 minutes at 37 °C.
Application: Critical for isolating fibroblasts, endothelial cells, and immune cells.
Challenge: Over-digestion can damage cell membranes and reduce viability.

Cryopreservation
Concept: Long-term storage of cells at ultra-low temperatures.
Related terms: freezing medium, DMSO, liquid nitrogen
Explanation: Cells are suspended in a cryoprotectant (usually 10 % DMSO) and cooled at ~-1 °C/min before storage.
Example: Freezing primary keratinocytes in 90 % FBS/10 % DMSO at –80 °C before transfer to liquid nitrogen.
Application: Allows banking of rare primary cell lines for future experiments.
Challenge: Ice crystal formation can cause membrane rupture; thawing must be rapid and controlled.

Culture Vessel
Concept: Container used to grow cells in vitro.
Related terms: flask, dish, multi-well plate
Explanation: Chosen based on cell type, surface area, and experimental needs; surface coating may be required.
Example: Using T-75 flasks coated with poly-L-lysine for neuronal cultures.
Application: Provides appropriate surface for cell attachment and proliferation.
Challenge: Inadequate gas exchange or surface coating can limit cell health.

Detergent-Based Lysis
Concept: Method to release intracellular components by solubilizing membranes.
Related terms: NP-40, Triton X-100, cell lysis buffer

Explanation: Utilized for downstream assays such as Western blotting; not a primary isolation technique but relevant for cell processing.
Example: Applying 0.1 % NP-40 to lyse isolated macrophages for protein extraction.
Application: Enables analysis of intracellular signaling pathways.
Challenge: Over-lysis can degrade proteins; insufficient lysis yields incomplete extraction.

Enzyme Cocktail
Concept: Combination of enzymes used to dissociate complex tissues.
Related terms: collagenase, dispase, hyaluronidase
Explanation: Tailored to tissue composition; balances efficient dissociation with preservation of surface markers.
Example: Using collagenase II (0.5 mg/mL) with dispase II (1 U/mL) for lung tissue.
Application: Improves yield of epithelial and mesenchymal cells from mixed organs.
Challenge: Variability between enzyme lots can affect reproducibility.

Epigenetic Profiling
Concept: Analysis of DNA methylation, histone modifications, and chromatin accessibility.
Related terms: ChIP-seq, bisulfite sequencing, ATAC-seq
Explanation: Provides insight into cell identity and differentiation status of primary cells.
Example: Performing ATAC-seq on freshly isolated cardiac fibroblasts to map open chromatin regions.
Application: Helps validate that isolated cells retain tissue-specific epigenetic signatures.
Challenge: Requires high-quality nuclei; low cell numbers can limit assay sensitivity.

Extracellular Matrix (ECM)
Concept: Network of proteins and polysaccharides surrounding cells in vivo.
Related terms: fibronectin, laminin, collagen
Explanation: Mimicking ECM components in culture (e.g., coating dishes) promotes appropriate cell adhesion and signaling.
Example: Coating plates with 10 µg/mL laminin for primary neuronal cultures.
Application: Enhances survival and functional maturation of delicate primary cells.
Challenge: Incorrect coating concentration can cause detachment or abnormal morphology.

Fetal Bovine Serum (FBS)
Concept: Common supplement providing growth factors, hormones, and attachment factors.
Related terms: serum-free medium, heat-inactivated serum, serum alternatives
Explanation: Typically added at 10–20 % v/v; batch variability can affect experimental outcomes.
Example: Adding 15 % FBS to a medium for primary smooth muscle cell expansion.
Application: Supports rapid proliferation of many primary cell types.
Challenge: Presence of undefined components may interfere with downstream assays; risk of viral contamination.

Flow Cytometry
Concept: Technique to analyze physical and fluorescent characteristics of individual cells.
Related terms: FACS, immunophenotyping, viability dye
Explanation: Enables sorting of specific cell subpopulations based on surface markers after isolation.
Example: Sorting $CD34^{+}$ hematopoietic stem cells from bone marrow using a FACSAria.
Application: Generates highly purified primary cell populations for functional studies.
Challenge: Requires single-cell suspension; clumping during dissociation can reduce sorting efficiency.

GentleMACS Dissociator
Concept: Mechanical device that standardizes tissue dissociation.
Related terms: rotor-stat, tissue grinder, automated dissociator
Explanation: Uses pre-programmed protocols to combine mechanical disruption with enzyme action, improving reproducibility.
Example: Processing mouse spleen with the “Mild” program and collagenase IV.
Application: Reduces operator variability in primary cell isolation.
Challenge: Initial equipment cost and need for specific disposable tubes.

Glucose Concentration
Concept: Amount of glucose present in culture medium, influencing cellular metabolism.
Related terms: high-glucose DMEM, low-glucose DMEM, glycolysis
Explanation: Primary cells often require physiological glucose levels (5–6 mM) to mimic in-vivo conditions.
Example: Using 5 mM glucose DMEM for cultured pancreatic islets.
Application: Prevents metabolic stress that can alter cell behavior.
Challenge: Inappropriate glucose can trigger unwanted differentiation or apoptosis.

Growth Factor Supplement
Concept: Recombinant proteins that stimulate proliferation or differentiation.
Related terms: EGF, FGF-2, insulin-like growth factor (IGF)
Explanation: Added at nanogram per milliliter concentrations; selection depends on cell lineage.
Example: Adding 20 ng/mL FGF-2 to maintain primary neural stem cells.
Application: Enhances expansion of otherwise slow-growing primary cells.
Challenge: Over-stimulation may lead to phenotypic drift or loss of tissue-specific markers.

Hank’s Balanced Salt Solution (HBSS)
Concept: Isotonic buffer used for washing and transporting tissues.
Related terms: PBS, RPMI, cell washing
Explanation: Maintains pH and osmolarity; often supplemented with calcium and magnesium for adhesion-dependent cells.
Example: Rinsing harvested skin tissue in cold HBSS before enzymatic digestion.
Application: Preserves cell viability during short-term handling.
Challenge: Prolonged exposure without nutrients can cause cell stress.

Hemocytometer
Concept: Manual counting chamber for estimating cell concentration.
Related terms: automated cell counter, viability dye, trypan blue
Explanation: Cells are mixed with trypan blue; viable cells exclude dye and are counted in defined grid squares.
Example: Determining a concentration of 1.2×10^6 viable cells/mL for seeding fibroblasts.
Application: Provides quick, low-cost cell density assessment.
Challenge: Subjective counting and limited throughput for large samples.

Immunomagnetic Separation
Concept: Use of antibody-coated magnetic beads to enrich specific cell types.
Related terms: MACS, CD markers, magnetic column
Explanation: Cells bound to beads are retained in a magnetic field, allowing purification of target populations.
Example: Isolating $CD45^+$ leukocytes from peripheral blood using anti-CD45 magnetic beads.
Application: Generates highly enriched primary immune cell subsets.
Challenge: Antibody binding can activate receptors, potentially altering cell function.

In-vitro Differentiation
Concept: Inducing lineage-specific maturation of primary progenitor cells in culture.
Related terms: stem cell, differentiation media, lineage markers
Explanation: Requires defined media, growth factors, and often extracellular matrix cues.
Example: Differentiating bone-marrow derived mesenchymal stem cells into adipocytes using insulin and dexamethasone.
Application: Produces functional cell types for disease modeling.
Challenge: Heterogeneous differentiation may lead to mixed cell populations.

Iso-osmotic Conditions
Concept: Maintaining equal osmolarity inside and outside cells to prevent swelling or shrinkage.
Related terms: osmolarity, tonicity, cell lysis
Explanation: Buffers like HBSS are formulated to be iso-osmotic (~300 mOsm/kg).
Example: Using iso-osmotic PBS during tissue rinses to avoid cell rupture.
Application: Protects delicate primary cells such as neurons during handling.
Challenge: Incorrect osmolarity can compromise membrane integrity.

Keratinocyte Growth Medium (KGM)
Concept: Specialized formulation supporting epidermal cell proliferation.
Related terms: calcium concentration, EGF, serum-free
Explanation: Contains low calcium (0.06 mM) and defined growth factors to promote keratinocyte expansion without serum.
Example: Culturing primary human keratinocytes in KGM for skin graft research.

Application: Enables reproducible epithelial cell culture.
Challenge: High calcium or serum contamination can induce premature differentiation.

Lactate Dehydrogenase (LDH) Assay
Concept: Colorimetric test measuring enzyme released from damaged cells.
Related terms: cell toxicity, viability assay, necrosis
Explanation: Increased LDH activity in supernatant indicates compromised membrane integrity.
Example: Monitoring LDH release after enzymatic digestion of lung tissue to assess cell damage.
Application: Provides rapid assessment of isolation protocol harshness.
Challenge: Background LDH from serum can confound results; requires serum-free controls.

Laminar Flow Hood
Concept: Enclosed workstation providing a filtered, unidirectional airflow.
Related terms: biosafety cabinet, HEPA filter, clean bench
Explanation: Protects cultures from airborne contaminants while shielding the operator from biohazardous material.
Example: Performing all media changes for primary hepatocyte cultures inside a class II hood.
Application: Standard equipment for aseptic primary cell work.
Challenge: Improper sash height or airflow disruption can reduce protection.

Live-Cell Imaging
Concept: Real-time visualization of cellular behavior using microscopy.
Related terms: time-lapse, fluorescence, incubation chamber
Explanation: Allows observation of migration, division, and morphological changes in freshly isolated cells.
Example: Tracking fibroblast migration on collagen-coated dishes over 48 hours.
Application: Evaluates functional competence of primary cells post-isolation.
Challenge: Phototoxicity and temperature fluctuations can affect cell health.

Mechanical Disaggregation
Concept: Physical methods (mincing, pipetting, vortexing) to break tissue into smaller fragments.
Related terms: tissue chopping, homogenizer, shear stress
Explanation: Often combined with enzymatic digestion to increase surface area for enzyme access.
Example: Mince cardiac tissue into ≤1 mm pieces before collagenase treatment.
Application: Enhances yield of viable cells from dense organs.
Challenge: Excessive force can cause cell membrane rupture and reduce viability.

Medium Supplement
Concept: Additive components that enhance cell growth beyond the basal formulation.
Related terms: serum, growth factors, antibiotics
Explanation: Tailored to cell type; may include hormones, trace elements, or antioxidants.
Example: Adding 10 µg/mL insulin to support primary adipocyte cultures.

Application: Optimizes conditions for specific primary cell lineages.
Challenge: Over-supplementation can mask deficiencies or introduce unwanted signaling.

Monolayer Culture
Concept: Growing cells as a single, contiguous sheet on a flat surface.
Related terms: confluent, subculture, passaging
Explanation: Most primary cells initially adhere as monolayers; density affects proliferation and differentiation.
Example: Maintaining primary endothelial cells at 70 % confluence to prevent contact inhibition.
Application: Facilitates easy observation and manipulation.
Challenge: Over-confluence can trigger senescence or loss of phenotype.

Mycoplasma Contamination
Concept: Infection by cell-wall-less bacteria that can alter cell behavior.
Related terms: PCR detection, antibiotic treatment, routine screening
Explanation: Often asymptomatic but can affect growth rates, metabolism, and experimental reproducibility.
Example: Performing PCR on DNA extracts from cultured primary fibroblasts to detect mycoplasma.
Application: Routine testing safeguards data integrity.
Challenge: Eradication may require prolonged antibiotic exposure, which can stress cells.

Neuronal Culture Medium (NCM)
Concept: Formulation designed to support primary neurons.
Related terms: B27 supplement, Neurobasal, glutamine
Explanation: Low in serum to prevent glial overgrowth; contains antioxidants and trophic factors.
Example: Culturing rat cortical neurons in Neurobasal medium supplemented with 2 % B27.
Application: Enables study of neuronal physiology and synaptic activity.
Challenge: Neurons are highly sensitive to osmotic shifts and require careful handling.

Optimum Seeding Density
Concept: Cell number per unit area that maximizes growth without causing stress.
Related terms: cell confluence, plating efficiency, passaging
Explanation: Determined experimentally for each primary cell type; influences proliferation and differentiation.
Example: Seeding primary keratinocytes at 5×10^4 cells/cm^2 to achieve rapid but healthy expansion.
Application: Ensures reproducible experimental conditions.
Challenge: Too low density leads to poor survival; too high leads to early contact inhibition.

Passage Number
Concept: Count of subculturing events a cell population has undergone.
Related terms: population doublings, senescence, phenotypic drift
Explanation: Primary cells have limited replicative capacity; higher passage numbers often correlate with loss

of original characteristics.
Example: Limiting fibroblast cultures to ≤5 passages before functional assays.
Application: Maintains tissue-specific functions and gene expression profiles.
Challenge: Balancing sufficient cell numbers with preservation of primary phenotype.

Permeabilization
Concept: Treatment that creates temporary pores in the plasma membrane.
Related terms: detergent, saponin, intracellular staining
Explanation: Used for immunocytochemistry to allow antibodies access to intracellular antigens.
Example: Using 0.1 % Triton X-100 to permeabilize primary astrocytes before staining for GFAP.
Application: Enables detection of intracellular markers in primary cultures.
Challenge: Over-permeabilization can cause loss of cytoplasmic proteins and cell morphology.

Phenotypic Characterization
Concept: Assessment of cell-type specific markers to confirm identity.
Related terms: immunostaining, flow cytometry, qPCR
Explanation: Involves detecting surface proteins, transcription factors, or functional enzymes.
Example: Verifying epithelial origin of isolated cells by staining for cytokeratin-18.
Application: Confirms successful isolation of the intended primary cell type.
Challenge: Marker expression may change rapidly in culture; timing of analysis is critical.

Plate Coating
Concept: Application of extracellular matrix proteins to culture surfaces.
Related terms: poly-L-lysine, Matrigel, collagen I
Explanation: Enhances attachment of cells that are otherwise non-adherent.
Example: Coating dishes with 10 µg/mL fibronectin for primary smooth muscle cells.
Application: Promotes proper morphology and function of delicate primary cells.
Challenge: Inconsistent coating can lead to uneven cell distribution.

Polymorphonuclear Leukocytes (PMNs)
Concept: Primary immune cells isolated from blood, including neutrophils.
Related terms: gradient centrifugation, Ficoll, dextran sedimentation
Explanation: Isolated using density gradients; used for functional assays such as chemotaxis.
Example: Isolating neutrophils via a two-step Percoll gradient for oxidative burst studies.
Application: Provides a source of innate immune cells for infection models.
Challenge: Short lifespan ex vivo requires rapid processing and use.

Protease Inhibitor Cocktail
Concept: Mixture of inhibitors that prevent proteolytic degradation during cell isolation.
Related terms: EDTA, PMSF, leupeptin
Explanation: Added to enzymatic digests to protect surface proteins and intracellular enzymes.

Example: Adding a cocktail containing 1 mM PMSF and 10 µg/mL leupeptin during tissue dissociation.
Application: Preserves antigenicity for downstream immunostaining.
Challenge: Some inhibitors may affect cell viability if concentrations are too high.

Quiescence Induction
Concept: Driving primary cells into a non-proliferative, resting state.
Related terms: serum starvation, contact inhibition, cell cycle arrest
Explanation: Achieved by reducing growth factors or reaching confluence; useful for synchronization studies.
Example: Culturing hepatic stellate cells in 0.5 % FBS for 48 hours to induce quiescence.
Application: Allows investigation of activation pathways upon re-stimulation.
Challenge: Prolonged quiescence can lead to phenotypic changes or senescence.

RNA Integrity Number (RIN)
Concept: Metric (0–10) indicating quality of extracted RNA.
Related terms: Bioanalyzer, mRNA stability, degradation
Explanation: High RIN (>8) is essential for reliable transcriptomic analyses of primary cells.
Example: Obtaining a RIN of 9.2 from freshly isolated mouse kidney cells before RNA-seq.
Application: Ensures accurate gene expression profiling.
Challenge: Delayed processing or harsh isolation can lower RIN.

Reagent Sterilization
Concept: Methods to eliminate microbial contaminants from solutions and tools.
Related terms: autoclave, filtration, UV irradiation
Explanation: Media are filtered through 0.22 µm membranes; heat-stable reagents may be autoclaved.
Example: Filtering prepared culture medium through a 0.22 µm syringe filter before use.
Application: Prevents introduction of bacteria or fungi into primary cultures.
Challenge: Some growth factors are heat-labile and lose activity if autoclaved.

Resazurin Assay
Concept: Colorimetric test measuring metabolic activity of viable cells.
Related terms: AlamarBlue, cell proliferation, redox indicator
Explanation: Viable cells reduce resazurin to fluorescent resorufin; signal correlates with cell number.
Example: Assessing viability of isolated chondrocytes after 24 hours using resazurin.
Application: Quick screening of isolation protocol toxicity.
Challenge: Serum components can interfere with fluorescence readout.

RNA-seq Library Preparation
Concept: Process of converting RNA into a sequencing-compatible format.
Related terms: cDNA synthesis, adapter ligation, amplification
Explanation: Requires high-quality RNA; low-input kits are available for scarce primary cell yields.

Example: Using a low-input kit to generate libraries from 10 ng of RNA from primary microglia.
Application: Enables comprehensive transcriptomic profiling of primary cells.
Challenge: Library bias can be introduced if RNA is degraded or fragmented.

Scaffold-Based Culture
Concept: Three-dimensional support that mimics tissue architecture.
Related terms: hydrogel, bioprinting, organoid
Explanation: Primary cells are embedded within biomaterials to promote more in-vivo-like behavior.
Example: Encapsulating hepatocytes in a collagen-Matrigel hydrogel for drug toxicity testing.
Application: Improves functional maturation and cell–cell interactions.
Challenge: Diffusion limitations can cause hypoxia in dense constructs.

Selective Adhesion
Concept: Exploiting differences in attachment kinetics to separate cell types.
Related terms: pre-plating, differential adhesion, cell sorting
Explanation: Certain cells adhere faster; short incubation allows removal of unwanted populations.
Example: Pre-plating a mixed lung cell suspension for 30 minutes to enrich for epithelial cells.
Application: Simple, cost-effective method for primary cell purification.
Challenge: Over-reliance may result in loss of slower-adhering target cells.

Serum-Free Medium (SFM)
Concept: Culture medium devoid of animal serum, defined by known components.
Related terms: defined medium, growth factor cocktail, supplement
Explanation: Reduces variability and eliminates serum-derived contaminants.
Example: Culturing primary endothelial cells in EGM-2 SFM supplemented with VEGF.
Application: Preferred for downstream applications requiring minimal background.
Challenge: Some primary cells cannot thrive without serum-derived attachment factors.

Shear Stress
Concept: Mechanical force exerted by fluid flow on cells.
Related terms: bioreactor, perfusion, laminar flow
Explanation: In microfluidic setups, shear can influence endothelial phenotype and alignment.
Example: Exposing primary arterial endothelial cells to 12 dynes/cm^2 to mimic arterial flow.
Application: Studies vascular mechanotransduction in a physiologically relevant context.
Challenge: Excessive shear can detach cells or cause apoptosis.

Single-Cell RNA-seq (scRNA-seq)
Concept: Transcriptomic profiling at the individual cell level.
Related terms: droplet microfluidics, barcoding, cell clustering
Explanation: Enables identification of heterogeneous subpopulations within primary isolates.
Example: Performing scRNA-seq on freshly isolated lung fibroblasts to reveal distinct activation states.

Application: Provides deep insight into cell heterogeneity and lineage trajectories.
Challenge: Requires viable single-cell suspensions; cell loss during dissociation can bias results.

Sialic Acid-Binding Lectin (SBL)
Concept: Protein used to isolate specific cell types based on surface glycans.
Related terms: lectin affinity, magnetic beads, cell sorting
Explanation: Lectins bind to carbohydrate structures unique to certain primary cells, facilitating enrichment.
Example: Using Sambucus nigra agglutinin-coated beads to isolate alveolar type II cells.
Application: Enables non-antibody based purification of delicate cells.
Challenge: Lectin binding may trigger signaling pathways affecting cell behavior.

Somatic Cell Reprogramming
Concept: Conversion of primary somatic cells into induced pluripotent stem cells (iPSCs).
Related terms: Yamanaka factors, retrovirus, episomal vectors
Explanation: Requires efficient delivery of transcription factors and careful culture conditions.
Example: Transducing primary fibroblasts with OCT4, SOX2, KLF4, and c-MYC to generate iPSCs.
Application: Provides patient-specific pluripotent cells for disease modeling.
Challenge: Low reprogramming efficiency and risk of genomic integration.

Spheroid Culture
Concept: Formation of three-dimensional cell aggregates without a scaffold.
Related terms: hanging drop, low-attachment plates, tumor spheroids
Explanation: Primary cells self-assemble, better recapitulating in-vivo cell–cell interactions.
Example: Generating breast epithelial spheroids from primary mammary tissue using ultra-low attachment plates.
Application: Useful for drug penetration studies and tumor biology.
Challenge: Nutrient diffusion limits size; central necrosis can develop.

Trypsin-EDTA
Concept: Enzymatic solution used to detach adherent cells.
Related terms: passaging, neutralization, cell detachment
Explanation: Trypsin cleaves adhesion proteins; EDTA chelates calcium to aid detachment.
Example: Incubating confluent fibroblast monolayers with 0.05 % trypsin-EDTA for 3 minutes at 37 °C.
Application: Facilitates subculturing and cell counting.
Challenge: Over-exposure can damage surface receptors critical for downstream assays.

Umbilical Cord Blood (UCB)
Concept: Source of primary hematopoietic and mesenchymal stem cells.
Related terms: $CD34^+$ cells, cord blood banking, stem cell transplantation
Explanation: Collected at birth; processed with density gradient centrifugation to isolate mononuclear cells.
Example: Isolating $CD34^+$ progenitors from UCB for in-vitro expansion.

Application: Provides a readily available, ethically uncomplicated primary cell source.
Challenge: Limited cell numbers per unit volume; requires efficient expansion protocols.

Viability Dye
Concept: Fluorescent molecule that discriminates live from dead cells.
Related terms: propidium iodide, 7-AAD, calcein-AM
Explanation: Membrane-impermeant dyes stain compromised cells; live cells exclude them.
Example: Using calcein-AM to label viable primary osteoblasts before flow cytometry.
Application: Immediate assessment of isolation success.
Challenge: Some dyes can be toxic at high concentrations; proper dilution is essential.

Wound Healing Assay
Concept: In-vitro method to study cell migration and repair.
Related terms: scratch assay, migration, closure rate
Explanation: A linear gap is created in a confluent monolayer; closure is monitored over time.
Example: Assessing fibroblast migration from a 500 µm scratch over 24 hours.
Application: Evaluates functional competence of primary cells after isolation.
Challenge: Edge effects and variability in scratch width can affect reproducibility.

X-ray Irradiation
Concept: Exposure of cells to ionizing radiation for experimental manipulation.
Related terms: DNA damage, radiosensitivity, dose-response
Explanation: Primary cells may be irradiated to study repair mechanisms or induce senescence.
Example: Subjecting primary lung epithelial cells to 2 Gy to assess DNA repair kinetics.
Application: Models radiation-induced injury in vitro.
Challenge: Primary cells often exhibit higher sensitivity than immortalized lines, requiring careful dose selection.

Yield Optimization
Concept: Strategies to maximize number of viable cells obtained from tissue.
Related terms: enzyme concentration, digestion time, tissue mincing
Explanation: Balances aggressive dissociation to increase yield with preservation of cell integrity.
Example: Adjusting collagenase concentration from 0.2 mg/mL to 0.5 mg/mL to improve cardiomyocyte recovery.
Application: Critical for scarce tissues such as human brain samples.
Challenge: Over-digestion can lead to loss of surface markers needed for downstream sorting.

Zero-Serum Media
Concept: Media formulations that contain no animal serum at all.
Related terms: defined medium, serum-free, chemically defined
Explanation: Relies on recombinant proteins, lipids, and hormones to support growth.

Example: Culturing primary retinal pigment epithelial cells in a chemically defined medium with insulin, transferrin, and selenium.
Application: Reduces variability for high-precision assays.
Challenge: Some primary cells cannot adapt and may undergo apoptosis without serum-derived attachment factors.