

Data Sheet

PAGE 1/3

BCRJ Code:	0428
Cell Line:	Hs-5
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Bone Marrow/Stroma
Morphology:	Fibroblast
Disease:	Normal
Growth Properties:	Adherent
Sex:	Male
Age/Ethnicity:	30 Year / White
Derivation:	Immortalization method: HPV-16 E6/E7 expression
Biosafety:	2
Additional Info:	HS-23 and HS-27A secrete low levels of growth factors and do not support proliferation of isolated progenitor cells in cocultures. The cell line can also be used as a feeder layer in ex vivo bone marrow cultures or in colony forming assays. HS-5 supports proliferation of hematopoietic progenitor cells when co-cultured in serum-deprived media with no exogenous factors
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose and fetal bovine serum to a final concentration of 10%.

Data Sheet

PAGE 2/3

Subculturing:

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 1.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. Place culture vessels in incubators at 37°C. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

Subculturing Medium Renewal:

Every 2 to 3 days

Subculturing Subcultivation Ratio:

1:3 to 1:9 is recommended

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

Data Sheet

PAGE 3/3

SAFETY PRECAUTION: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

Roecklein BA, Torok-Storb B. Functionally distinct human marrow stromal cell lines immortalized by transduction with the human papilloma virus E6/E7 genes. Blood 85: 997-1005, 1995. PubMed: 7849321 Torok-Storb B, et al. Human marrow stromal cell lines which sustain hematopoieses. US Patent 5,879,940 dated Mar 9 1999

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Thawing Frozen Cells:

References:

Depositors:

