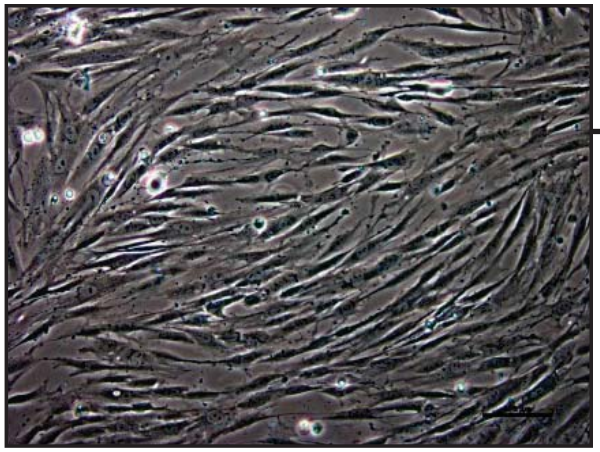

Human Embryonic Progenitor Cell Line 4D20.8

Cat No. ES-84

The clonal human embryonic progenitor cell line 4D20.8 was derived from the registered parental hES cell line H9 (WA09) as described (West et al, 2008). It displays markers of cranial neural crest mesechyme. Distinguishing it from other neural crest lines is relatively high expression levels of *BARX1* and *LHX8*. *BARX1* is implicated in the regulation of molariform dentition in the maxillary and mandibular ectomesenchyme (Mitsiadis, TA et al, 1998). *LHX8* is a marker of maxillary (secondary palate) and mandibular mesenchyme (Zhang, Y et al, 2002). When differentiated with the kit ES-K42, the line exhibits chondrogenic potential *in vitro* and a relative lack of hypertrophic chondrocyte gene expression markers characteristic of bone marrow mesenchymal stem cells such as *COL10A1*, *IHH* or the MSC marker *CD74*.

Positive mRNA Markers: *BARX1, LHX8, BBOX1, FGF18, LINGO2, MSX2*

Product Information:

Derivation: Progenitor line 4D20.8 was derived from the parental NIH registered human ES cell line H9 (WA09) as previously described (West, M.D. et al, 2008).

Cell Number: Vial contains >500,000 cells cryopreserved in 1 ml of FBS with 10% DMSO.

Recommended Growth Medium: ES-N170

Recommended Culture Conditions: Cells should be plated onto tissue culture grade polystyrene plastic coated with 0.1% gelatin. Following rapid thawing and slow dilution in the final culture medium, the initial seeding density should be approximately 20,000 cells/cm^2. Cells should be maintained at 37°C in a humidified incubator preferably with 5% CO_2 and 5% oxygen with media change at least twice per week. Upon reaching confluence they should be split 1:3 for routine maintenance. Note: confluence for more than two days may lead to terminal differentiation.

Available Differentiation Kits:

- **Chondrogenesis Differentiation Kit:** Cat No. ES-K42

Population Doubling Time: Approximately 50 hours.

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Population Doubling: Progenitor line 4D20.8 is sold at passage 11 (original clonal isolate in confluent 1.9 cm^2 well being P1), which corresponds to approximately 13.5 doublings since the first 1.9 cm^2 well and approximately 31.5 doublings since its original clonal plating. The line displays a finite lifespan in vitro, senescing at approximately 70 PD. When used properly this product should scale for a minimum of 10 population doublings.

Quality Control:

Sterility: The line is negative for HIV (1, 2), HBV, HCV, EBV, bacteria, mycoplasma, and fungal contamination.

Thaw Test Result: >70% viability, >80% attachment, growth to confluence, and maintenance of original morphologic appearance.

Certificates of Analysis: Available on request.

Restrictions: These cells are provided for research purposes only. They are not for human use, and may not be used for commercial purposes. The user is responsible for proper handling upon receipt.

Technical Assistance: Please contact a representative at ES Cell International (support@biotimemail.com or (510) 521-3390) for technical assistance.

Pricing:

Cat Number	Description	List Price
ES-84	4D20.8 Embryonic Progenitor Cell Line	$2,800.00
ES-N170	ESpan Cell Culture Medium Kit (with supplements)	99.00
ES-K42	Chondrogenesis Differentiation Kit	295.00

References:

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West, M.D., Sargent, R.G., Long, J., Brown, C., Chu, J-S., Kessler, S., Derugin, N., Sampathkumar, J., Burrows, C., Vaziri, H., Williams, R., Chapman, K.B., Larocca, D., Loring, J.F., and Murai, J. 2008. The ACTCellerate Initiative: large-scale combinatorial cloning of novel human embryonic stem cell derivatives. *Reg. Med.* 3(3): 287-308.

Zhang, Y., Mori, T., Takaki, H., Takeuch, M., Iseki, K., Hagino, S., Murakawa, M., Yokoya, S., and Wanaka, A. 2002. Comparison of the expression patterns of two LIM-homeodomain genes, Lhx6 and L3/Lhx8, in the developing palate. *Orthod. Craniofac. Res.* 5:65-70.