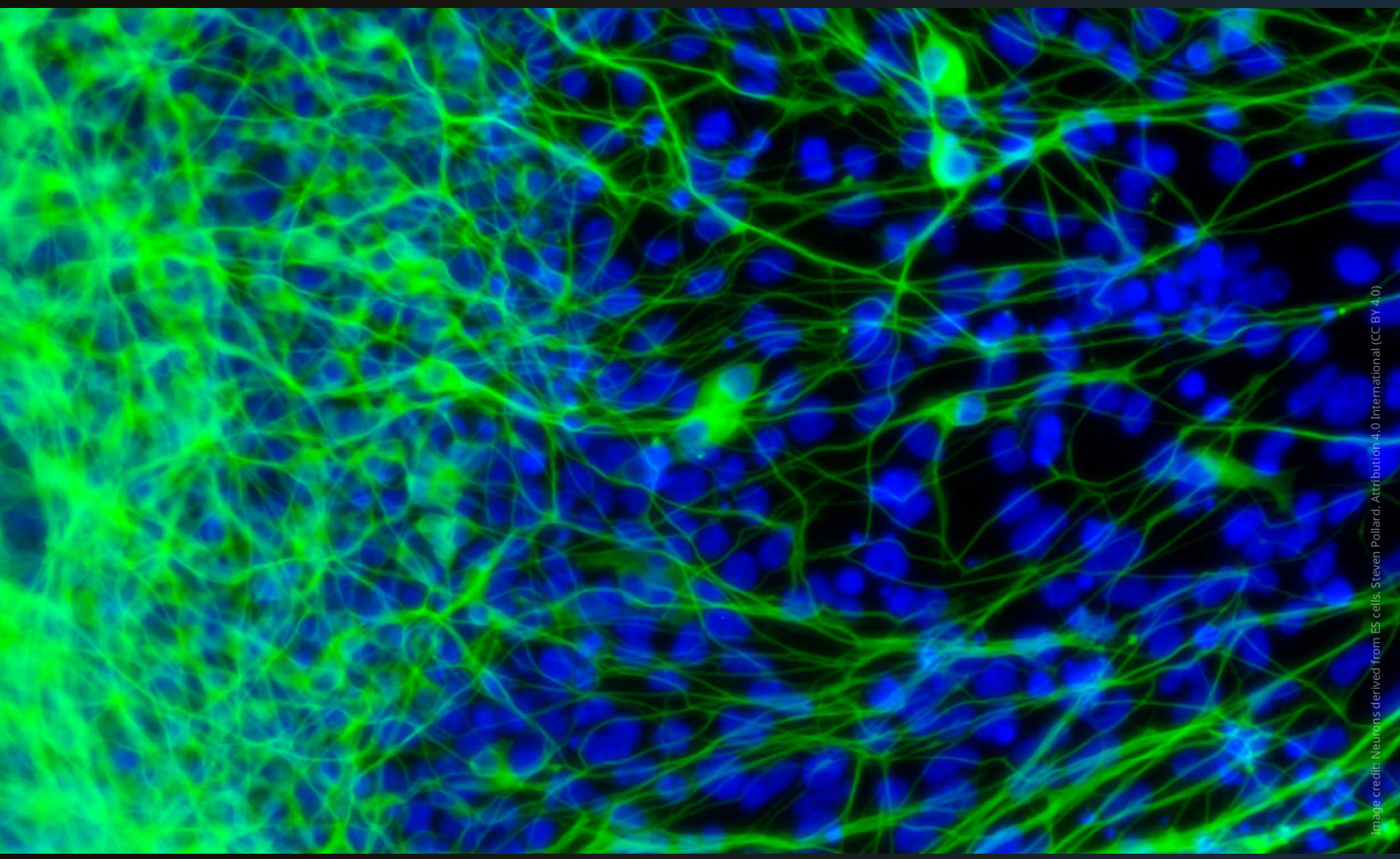
Qkine

Growth factors for

Neural

and glial cell differentiation



Enhanced reproducibility

Protein innovation

Bioactivity. Guaranteed.

Manufactured in Cambridge, UK

Improved growth factors for neural stem cell and organoid culture

High purity reliable growth factors and cytokines are essential for the generation and maintenance of robust, reproducible and physiologically relevant neural cell populations. Selecting animal origin-free proteins for sensitive stem cell differentiation protocols reduces uncertainty by preventing contamination from co-purified endogenous growth factors and facilitates translation to pre-clinical and high throughput applications.

Qkine manufactures highly bioactive, exceptional purity, animal origin-free recombinant proteins at an ISO9001:2015 certified facility in Cambridge, UK. All growth factors and cytokines are manufactured within a stringent quality framework and adhere to our Nine-point Quality Commitment, including full detailed quality control testing on every lot to ensure no lot-to-lot variation in protein quality or EC50. As part of our Bioactivity Guarantee, we conduct comparative quantitative bioactivity studies with dominant suppliers to ensure the bioactivity of all proteins is equivalent or greater. Higher compliance product documentation, including detailed lot-specific CoO, CoA, and animal-free certification are available for translational studies.

We combine proprietary production processes with protein engineering technology to tackle fundamental biological, quality, and scale-up challenges to provide more reliable tools for research and bio-manufacturing, including new animal origin-free proteins, thermostable and protein tag-free growth factors and related proteins.

Raising the standard in bioactive protein manufacturing and innovation

Manufactured in Cambridge, UK

Animal-free and carrier-protein free

In stock for fast worldwide delivery

Bulk quantities available for large scale applications

High purity and protein tag-free

ISO 9001:2015 accredited facility

Highly bioactive

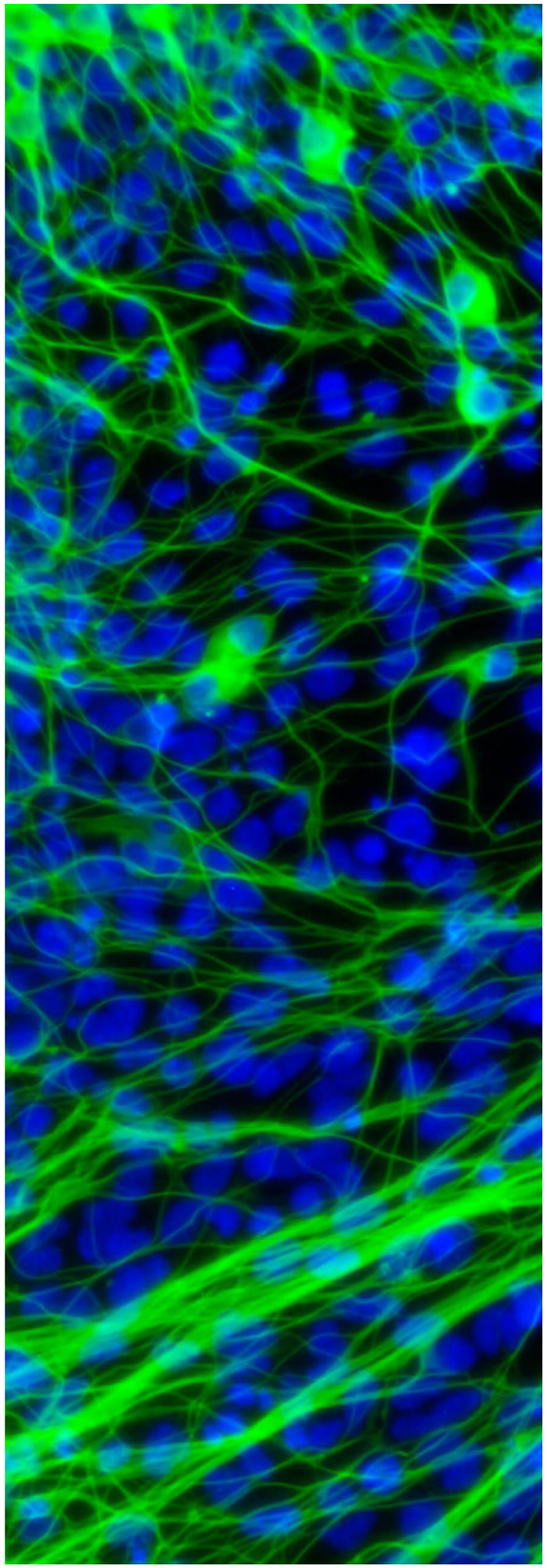
Innovative engineered protein forms available

Neural stem cells

Neural stem cells (NSCs) are multipotent stem cells that can differentiate into cells within the neural lineage. NSCs give rise to committed progenitors which are neuronal or glial cell progenitors. These committed progenitors have the capacity to proliferate and differentiate into specific specialized cells but their self-renewal is limited. Neuronal progenitors give rise to all the different types of neurons, the basic functional unit of the nervous system. Glial progenitors give rise to supporting cells such as oligodendrocytes, astrocytes, and Schwann cells.

Neurogenesis and NSCs are regulated by intrinsic genetic and epigenetic factors and by extrinsic stimuli. Disruptions in those factors leads to age related cognitive disorders including Alzheimer's and Parkinson's diseases; neurological and psychiatric disorders, including depression and schizophrenia; and cancer. These diseases have been traditionally difficult to research given the complexity of neural tissue and the paucity of suitable *in vitro* model systems. This has led to a significant rise in interest in NSCs as model systems to study neural development, disease mechanisms, and drug screening. Several promising new cell therapy strategies also employ stem cell-derived neurons or glial cells.

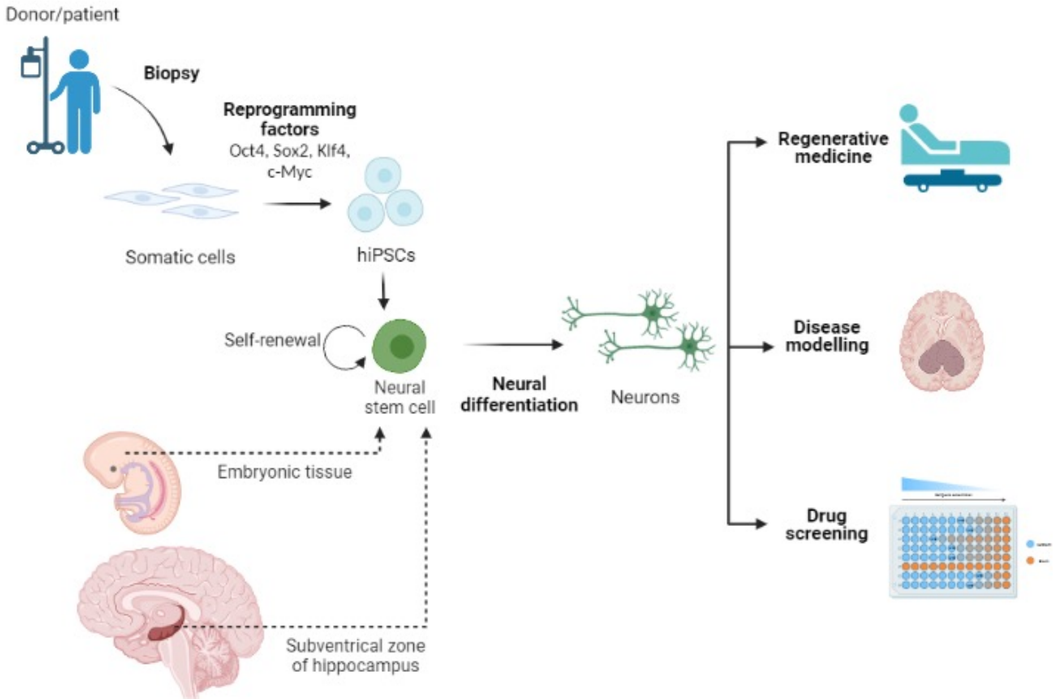
> "BDNF and GDNF are used daily in a neuronal culture lab, and the BDNF and GDNF from Qkine has provided cost-effective and bioactive proteins to produce human motor neurons and maintain their health overtime."
>
> Joshua Thomas and Felix Buchner, DZNE German Center for Neurodegenerative Diseases



Neural induction and stem cell maintenance

Neural induction is the initial step to drive induced pluripotent stem cells (iPSCs) or embryonic stem cells (ESCs) to a neuronal lineage. Firstly, iPSCs or ESCs are cultured in media supplemented with fibroblast growth factor-2 (FGF-2) and other growth factors until they are confluent, then passaged on to the Matrigel or another defined matrix to derive NSCs and neural progenitor cells. Standard protocols include bone morphogenetic protein (BMP) inhibitors such as noggin, small molecule SMAD inhibitors such as dorsomorphin and Wnt inhibitors such as DKK-1 to inhibit signaling pathways that promote non-neural lineages.

FGF-2 and epidermal growth factor (EGF) are the main growth factors used to maintain the pluripotency of NSCs in culture. A thermostable FGF-2, such as FGF2-G3 (Qk053), can be used for weekend-free stem cell maintenance or to improve homogeneity of cells prior to differentiation to increase final specific cell-type yield. Other growth factors such as vascular endothelial growth factor (VEGF) or nerve growth factor (NGF) may promote the proliferation and survival of neural stem cells.



Product code	Description	Product code	Description
Qk001	Human/mouse Activin A	Qk053	Thermostable FGF2-G3 154 aa
Qk005	Human/mouse Activin A PLUS	Qk046	Human KGF (FGF-7)
Qk035	Follistatin-resistant Activin A	Qk003	Human/rat/bovine/porcine FGF-10
Qk038	Human BMP-4	Qk013	Human HGF (NK1)
Qk011	Human EGF protein	Qk049	Human Oncostatin M (OSM) protein
Qk025	Human FGF-2 (bFGF) 145 aa	Qk031	Human R-spondin 1 LR5
Qk027	Human FGF-2 (bFGF) 154 aa	Qk006	Human R-spondin 1
Qk052	Thermostable FGF2-G3 145 aa	Qk032	Human R-spondin 3

Neural differentiation with Dickkopf-related protein 1 (DKK-1)

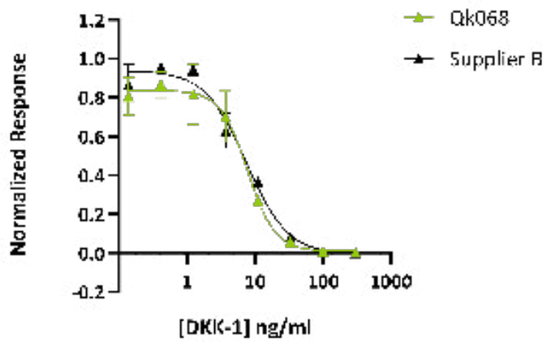
Qkine produce the only commercially available animal-free bioactive DKK-1 for reproducible stem cell culture and related applications

Dickkopf-related protein 1 (DKK-1) is a potent Wnt pathway antagonist used to control cell fate, self-renewal, and differentiation. Recombinant human DKK-1 is used in stem cell differentiation protocols, particularly in neural and osteogenic pathways, and for mimicking dysregulated Wnt signaling seen in cancers. Qkine has recently developed a fully animal-origin free (AOF) DKK-1 to support translational and sensitive studies. Using high-purity AOF proteins improves reproducibility by eliminating contamination or off-target effects from trace animal components and co-purifying related proteins naturally secreted by mammalian protein expression systems.

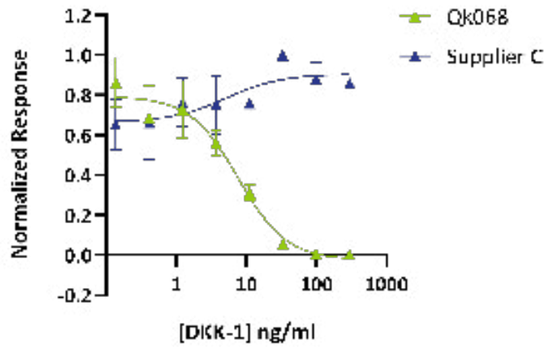
To determine relative bioactivity of mammalian and bacterially expressed DKK-1, the bioactivity of Qkine AOF DKK-1 (Qk068) was compared directly with mammalian-expressed DKK-1 from a main supplier in a Wnt-3a-responsive firefly luciferase reporter assay. The bioactivity of these proteins was comparable (a).

Qkine DKK-1 activity was also compared with a commercially available bacterially expressed DKK1. This protein showed no inhibition of Wnt activity in the reporter assay, suggesting DKK-1 from the alternative supplier is not biologically active (b).

a.



b.



Quantitative luciferase reporter assay shows equivalent bioactivity of Qkine AOF DKK-1 and mammalian-expressed DKK-1.

HEK293T cells were treated in triplicate with a serial dilution of DKK-1 and a standard concentration of Wnt-3a for 24 hours. a. bioactivity of bacterially-expressed AOF DKK-1 (Qkine, green Qk068) and mammalian-expressed DKK-1 (Supplier B, black, Peprotech 120-30) was observed with an EC50 of 7.8ng/ml (301pM) and 7.5ng/ml (291pM) respectively. b. No biological activity was determined using bacterially-expressed DKK-1 from an alternative supplier (Supplier C, blue, ProSpec PRO-1566). Please note the supplier does not provide information on the biological activity of this protein.

The comparative bioactivity data demonstrate that Qkine DKK-1 (Qk068) has equivalent bioactivity to the mammalian-expressed DKK-1 from an alternative major supplier. Qkine thus provides the first reliable source of high-quality animal-free DKK-1 for highly-defined reproducible culture of stem cells, neuronal cells, osteoblasts and other applications.

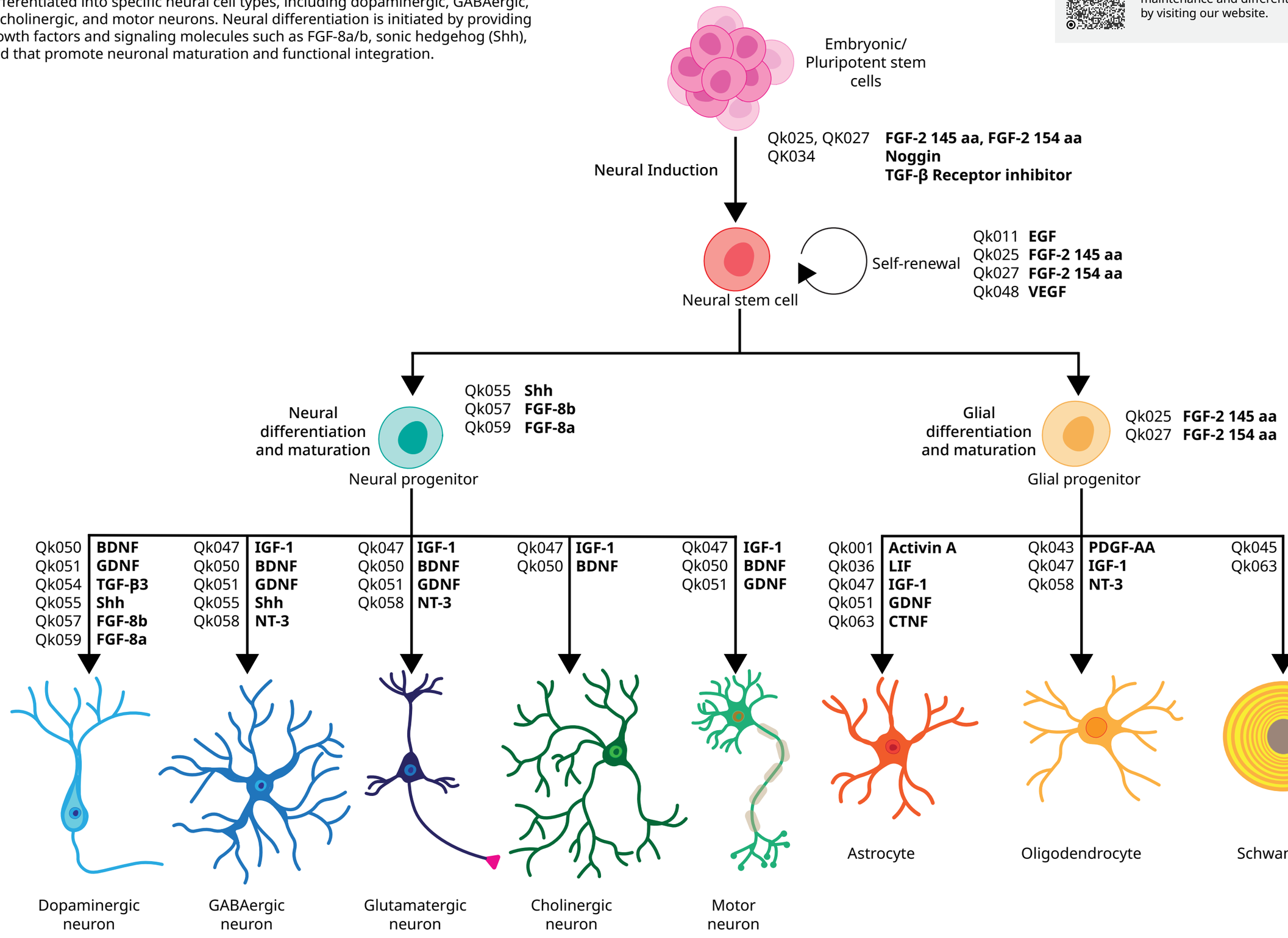
Learn more about ultra high-purity, animal origin-free Qkine DKK-1.

Product code	Description
Qk068	Recombinant human DKK-1

Neural differentiation

NSCs can be differentiated into specific neural cell types, including dopaminergic, GABAergic, glutamatergic, cholinergic, and motor neurons. Neural differentiation is initiated by providing appropriate growth factors and signaling molecules such as FGF-8a/b, sonic hedgehog (Shh), and retinoic acid that promote neuronal maturation and functional integration.

Learn more about neural and glial cell maintenance and differentiation by visiting our website.

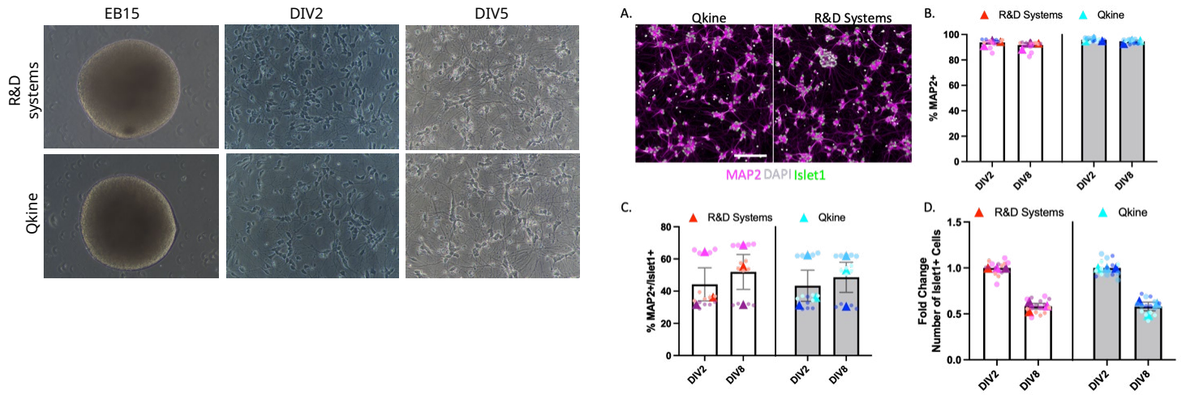


BDNF and GDNF neural growth factors for enhanced iPSC-derived neuronal cultures

Brain-Derived Neurotrophic Factor (BDNF) and Glial Cell-Derived Neurotrophic Factor (GDNF) are part of the neurotrophic factor family that play a crucial role during embryonic development and the maintenance of the nervous system during adulthood. In cell culture, they are essential growth factors in the differentiation of iPSCs into motor neurons and maintenance of the distinctive characteristics and functions of cultured neurons over prolonged durations. Therefore, it is essential to have high-quality and bioactive proteins to generate reproducible, reliable, and physiologically relevant neural cultures.

- Recombinant human BDNF and GDNF from Qkine (both AOF) and R&D systems (Baculovirus/mouse myeloma derived) were compared for their ability to differentiate iPSCs embryoid bodies (EB15) into motor neurons. Morphology and viability were comparable after 2 (DIV2) and 5 (DIV 5) days.

- Expression of motor neuron markers microtubule-associated protein 2 (MAP2) and Islet1 confirmed differentiation of the iPSCs with 95% of the cells expressing MAP2 and 40-50% Islet1. Expression was equivalent between Qkine AOF BDNF and GDNF and Baculovirus/mouse myeloma derived growth factors.



Morphological and viability assessment of embryoid bodies and dissociated motor neurons cultured with BDNF and GDNF.

Brightfield images representing EBs at day 15 (EB15) of differentiation protocol (scale bar = 250µm) and dissociated MNs from the same EBs after 2 (DIV2) and 5 (DIV5) days *in vitro* (scale bar = 100µm). Immunocytochemistry images representing iPSC-derived motor neurons stained with DAPI (grey), MAP2 (magenta), and Islet1 (green) at DIV8 (A, scale Bar = 50µm). Quantification of the percentage of MAP2+ neurons (B), percentage of MAP2+/Islet1+ cells (C), fold change of the number of Islet1+ cells after DIV2 and DIV8 (D).

Recombinant human BDNF and GDNF proteins from both Qkine and R&D systems effectively generated and maintained healthy iPSC-derived MNs in both 2D and 3D culture systems. Animal-free proteins reduce variability from animal derived materials and ensure a more controlled environment and reproducibility in the differentiation of MNs.

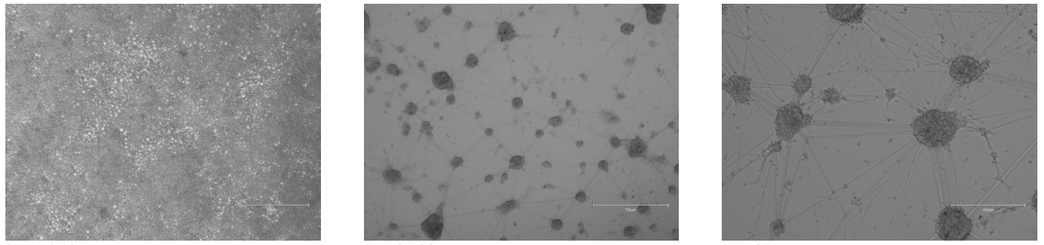
Learn more about recombinant BDNF and GDNF proteins from Qkine by reading our application note.

Differentiation of induced pluripotent stem cells (iPSCs) into dopaminergic neurons

The dopaminergic system is known to play an important role in neuromodulation processes, such as cognitive function, motor control, motivation, and reward behaviors. Dopaminergic signaling pathways are therefore crucial in the maintenance of physiological processes and dysfunctions of these pathways have been implicated in several neuropsychiatric and neurodegenerative diseases, such as Parkinson's disease and Schizophrenia. Dopaminergic neurons are a major source of dopamine produced in the mammalian central nervous systems.

- iPSCs were differentiated into dopaminergic neurons using sequential media changes with the addition of Qkine fibroblast growth factor 8a (FGF-8a, Qk059), brain-derived neurotrophic factor (BDNF, Qk050), glial cell line-derived neurotrophic factor (GDNF, Qk051) and transforming growth factor (TGFb3, Qk054).

- Mature dopaminergic neurons could be visualized within 35 days.



Day 14-19 | Day 21-30 | Day 35

Maturation of dopaminergic neurons.

Day 14-19, midbrain-specified floor plate progenitor cells differentiating into floor plate spheres before sphere isolation passage. Day 21-30, differentiating dopaminergic neurons. Day 35, matured dopaminergic neurons.

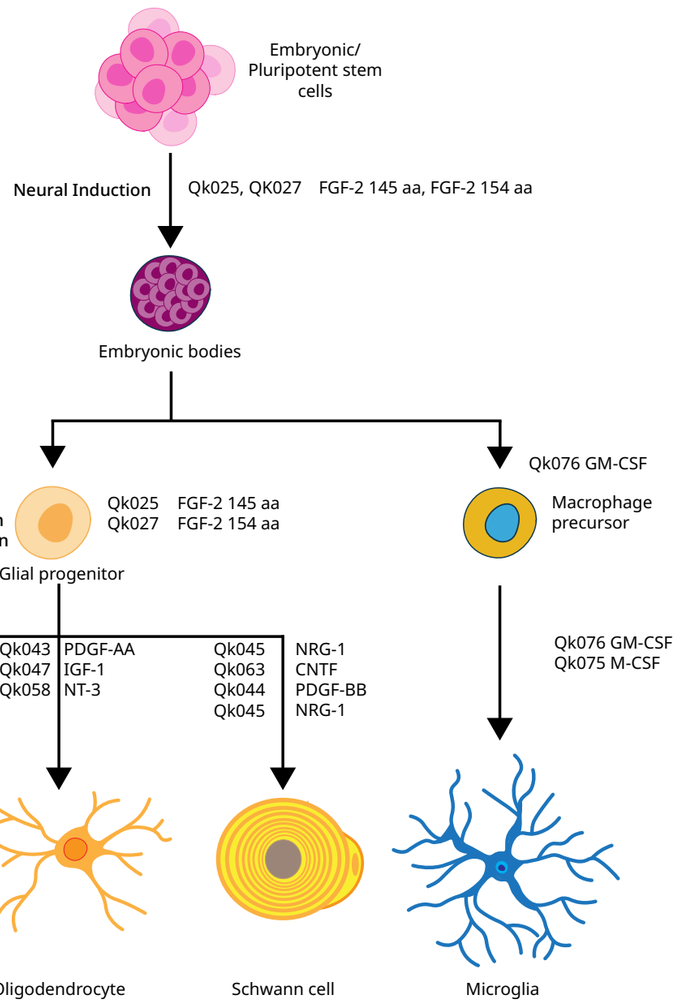
Day 1	Induction media 1	Knockout DMEM with 15% serum replacement, glutamax, non-essential amino acids (NEAA), 10µM 2-mercaptoethanol (2-ME), 100nM LDN193189 and 10µM SB431542
Day 2 + 3	Induction media 2	Induction media 1 + 100nM SAG hydrochloride, 2µM puromorphamine and 100ng/ml Qkine FGF8a (Qk059)
Day 4 + 5	Induction media 3	Induction media 2 + 3µM CHIR99021
Day 6 + 7	Induction media 4	3:1 knockout DMEM with 15% serum replacement: neurobasal media (NB) with glutamax, NEAA, N-2 and B-27 supplements, 10µM 2-ME, 100nM LDN193189 and 10µM SB431542,100nM SAG hydrochloride, 2µM puromorphamine, 3µM CHIR99021 and 100ng/ml Qkine FGF8a
Day 8 + 9	Induction media 5	1:1 knockout DMEM with 15% serum replacement: NB with glutamax, NEAA, N-2 and B-27 supplements, 10µM 2-ME, 100nM LDN193189 and 3µM CHIR99021
Day 10 + 11	Induction media 6	1:3 knockout DMEM with 15% serum replacement:NB with glutamax, NEAA, N-2 and B-27 supplements, 10µM 2-ME, 100nM LDN193189 and 3µM CHIR99021..
Day 12 + 13	Induction media 7	NB with glutamax, N-2 and B-27 supplements, 3µM CHIR99021, 10µM DAPT, 500 µM N6,2'-O-Dibutyrladenosine 3':5'- cyclic monophosphate sodium salt, 200µM Ascorbic acid, 20ng/ml BDNF (Qk050), 20ng/ml GDNF (Qk051) and 1ng/ml TGFb3 (Qk054).
Day 14 +	Maturation media	NB with glutamax, B-27 supplement, 10µM DAPT, 500µM N6,2'-O-Dibutyrladenosine 3':5'-cyclic monophosphate sodium salt, 200µM Ascorbic acid, 20ng/ml BDNF, 20ng/ml GDNF and 1ng/ml TGFb3.

Access the full media components and protocol from Qkine by reading our application note.

Glial cell differentiation

Once thought to be filler cells for the central nervous system, glial cells are now understood to have vital functions in the regulation of neurotransmission. Dysfunction in glial cells is associated with diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, glioblastoma, autism, and psychiatric disorders. There is intense interest in neural stem cell differentiation to glial lineages, which requires the presence of growth factors EGF and FGF-2. Stem cell-derived models allow the study of the complex role of glial cells, including microglia in disease and aging providing unique insights into basic biological mechanism.

Glial progenitors can be differentiated into several supporting cells including astrocytes, oligodendrocytes, and Schwann cells. Astrocytes have a critical role in neurotransmitter homeostasis, regulation of blood flow and synapse function. Oligodendrocytes and Schwann cells myelinate axons, ensuring efficient signal conduction and preserving axon structure.



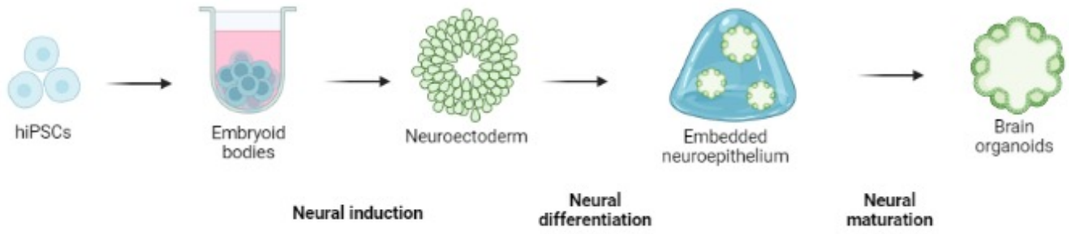
3D cell-based models of the brain: brain organoids, neurospheres, organ-on-chip and bio-printing

Adherent neural monolayers do not fully recapitulate the complexity of the human brain, however when combined with other cell types and microfluidic devices their functionality and relevance is enhanced, the relative simplicity and reproducibility of the system provides utility is drug screening and high-throughput applications.

Neurospheres, composed of clusters of NSCs or neural progenitor cells (NPCs) cultured in suspension in serum-free media supplemented with EGF or FGF-2, provide a degree of biological complexity to investigate the regulation of self-renewal and differentiation processes and have uses in drug development or in identification of environmental factors that modulate neural cell fate.

Recent advancements in tissue engineering, with both spontaneous formation of brain organoids, or more directed approaches using soluble growth factors, extracellular matrices, and other cues have led to successively more complex in vitro 3D models such as organoids. New approaches including details patterning, bio-printing and co-culture with immune cells are at the frontier of disease modelling and understanding developmental biology. As the complexity and length of neural cell culture protocols extends to push the frontiers of science, the reliability and reproducibility of growth factors and other cell culture reagents is critical. At Qkine, we are committed to raising the standards of growth factors, cytokines and related proteins to better support long-term and complex neural stem cell culture. We are a science-led team, please reach out with any questions or requests to support@qkine.com

Diagram and product list for brain organoids



Product code	Product description
Qk001	Recombinant human/mouse/rat/bovine/porcine Activin A protein
Qk005	Recombinant human/mouse/rat Activin A PLUS protein
Qk011	Recombinant human EGF protein
Qk018	Recombinant mouse LIF protein
Qk025	Recombinant human FGF-2 145 aa protein
Qk027	Recombinant human FGF-2 154 aa protein
Qk033	Recombinant mouse/rat noggin protein
Qk034	Recombinant human noggin protein
Qk035	Recombinant human/mouse/rat follistatin-resistant Activin A protein
Qk036	Recombinant human LIF protein
Qk043	Recombinant human PDGF-AA protein
Qk044	Recombinant human PDGF-BB protein
Qk045	Recombinant human/bovine/porcine NRG-1 protein
Qk047	Recombinant human/bovine/porcine IGF-1 protein
Qk048	Recombinant human VEGF 165 protein
Qk050	Recombinant human BDNF protein
Qk051	Recombinant human GDNF protein
Qk052	Recombinant FGF2-G3 145 aa protein
Qk053	Recombinant FGF2-G3 154 aa protein
Qk054	Recombinant human TGF-β3 protein
Qk055	Recombinant human Shh protein
Qk057	Recombinant human mouse FGF-8b protein
Qk058	Recombinant human/mouse/rat/porcine NT-3 protein
Qk059	Recombinant human/mouse FGF-8a protein
Qk063	Recombinant human CNTF protein

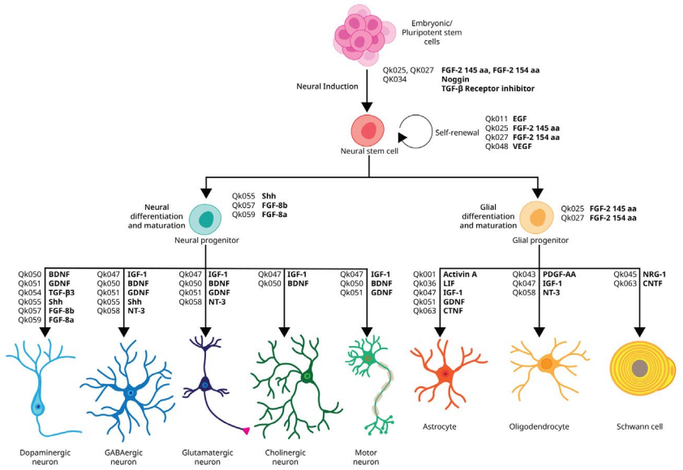
More resources at qkine.com

Qkine

Neural and glial cell maintenance and differentiation

Growth factors required to derive human neural cell types from induced pluripotent stem cells

qkine.com/neural-stem-cell-culture/



Reconstituting lyophilized proteins

Qkine growth factors are lyophilized to maintain biochemical quality, improve stability, and allow shipping at ambient temperatures to enhance sustainability.

Quick calculator

The optimum reconstitution solution for each protein is determined experimentally.

Reconstitute to a concentration of >50-1000 µg/ml, dilute in sterile physiological buffer as required, prepare single-use aliquots and store frozen.

Reconstitution calculator

Mass in vial (µg) ÷ Desired concentration (µg/ml) X 1000 = Volume to add (µl)

We're happy to help, please email support@qkine.com, or visit qkine.com/your-proteins

We're here to help you achieve successful and stress-free science
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Stringent quality control at every step

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Raising the standard in bioactive protein manufacturing and innovation

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or email customerservice@qkine.com

Qkine products are for research use and ex vivo cell manufacturing use only

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