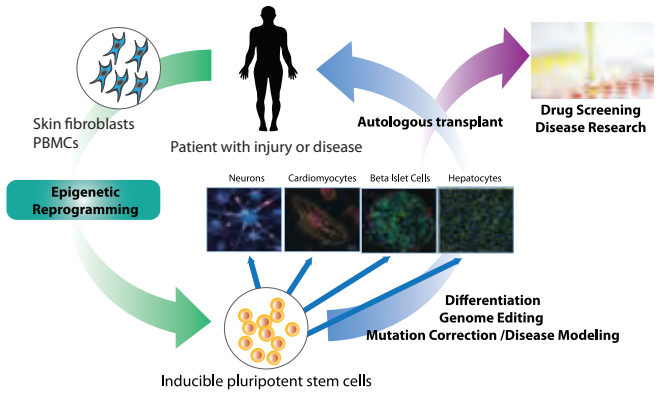
Applied StemCell

iPSC Generation, Disease Modeling & Correction, Differentiation and Neurotoxicity Screening

One-stop solution for all your stem cell needs; affordable, high quality services and products for disease modeling & drug screening

Why work with ASC?

- **Global leader** in stem cell and gene editing technologies
- **ISO 9001 and ISO 13485 certified** quality management system
- **In-licensed** iPSC and CRISPR/Cas9 technologies
- **Flexible project modules** to customize service deliverables to fit your specific requirements
- **Complete toolset of services and products** for every aspect of your stem cell needs
- **Comprehensive test battery for neurotoxicity and CNS drug screening**



Contents of this brochure:

- iPSC generation (p1)
- iPSC Gene Editing (p2)
- iPSC Characterization (p4)
- iPSC Differentiation (p5)
- IPSCs, Neural Stem Cell, Neural Cells; other related cell lines (p6)
- Neurotoxicity Screening (p7)
- iPSC Cell Culture Products (p8)

Footprint-Free iPSC Generation Service

ASC has successfully reprogrammed hundreds of high quality iPSCs from both healthy and disease patient samples, and other mammalian species, using in-licensed reprogramming technology from iPSC Academia (Japan) and highly optimized proprietary protocols.

- iPSC reprogramming from PBMC, fibroblasts, CD34+ blood cells
- Integration-free reprogramming: suitable for drug discovery and cell therapy applications
- High reprogramming efficiency
- Feeder-free culture protocols

Services includes:
- Cell recovery - Transfection with reprogramming vectors - Single cell cloning and expansion - Characterization of iPSCs with customizable options

Deliverables and Timeline:
- At least two clones with 2 vials per clone - Characterization of iPSCs with customizable options - Dedicated project managers; milestone and final report **Timeline:** 2-3 months

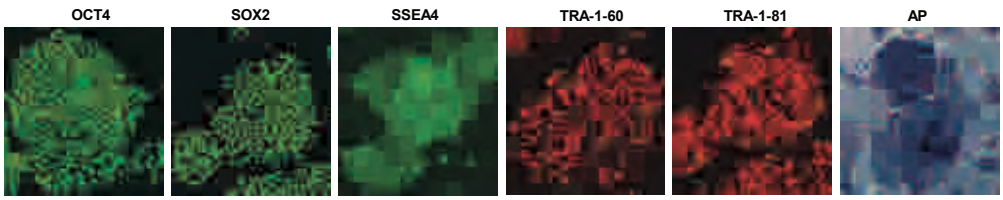


Figure: Human iPSCs were generated from dermal fibroblasts from a patient with a rare disorder, using feeder-free, proprietary protocols. The reprogrammed iPSC clones were characterized by pluripotency marker staining for OCT4, SOX2, SSEA4, TRA-1-60, TRA-1-81, and alkaline phosphatase (AP).

iPSC Genome Editing/ Disease Modeling Services

ASC's has extensive expertise in both iPSC and CRISPR/Cas9 gene editing technologies, and is uniquely placed to provide the best iPSC genome editing/ disease modeling services. With our extensive expertise we have generated hundreds of distinct mutations in various iPSC lines with a more than 98% success rate.

- Two highly efficient gene editing technologies: CRISPR/Cas9 and TARGATT™
- Genome editing in iPSCs & stem cells from healthy and disease patients; and other mammalian species
- Wide range of control and patient iPSC lines available for disease modeling and therapeutic research
- Customizable deliverables

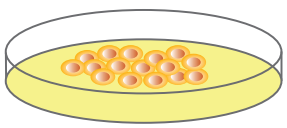
Gene knockout

Gene Insertion/ Knock-in
(reporter gene, small fragment/ point mutation)

Gene correction or replacement

Inducible expression or overexpression

iPSC Gene Modification



- **CRISPR/Cas9**
- **TARGATT™**

ASC provides services under licensing agreement with the Broad Institute for CRISPR/Cas9 Gene-Editing technology and iPS Academia, Japan for iPSC technology along with ASC's proprietary TARGATT™ technology.

Service includes:

- Cell line validation
- Target DNA vector construction and validation
- Transfection of CRISPR/Cas9 constructs
- Gene editing confirmation and clonal expansion
- Pluripotency characterization after genome editing (optional)

Deliverables and Timeline

- Genetically engineered iPSCs with confirmed mutations
- Customizable deliverables:
  - Choice of heterozygous and homozygous mutations
  - Gene editing without silent mutations (for critical research areas)
  - Feeder-free or feeder-dependent protocols
  - Footprint-free or lentivirus-based gene editing
  - Pluripotency characterization after gene editing
  - Differentiation into terminal lineage cells

Timeline: as little as 3 months

New! Lentiviral Stable Cell Line Generation Service
(Integrating & non-integrating lentiviruses available)
Ask for details.

Benefits and Applications:

- Unlimited resource of *in vitro* models
- Isogenic control and disease cell lines for reliable comparison of results
- Physiologically relevant disease models for hard-to-model diseases
- Ideal for disease modeling, developmental genetics research, target drug discovery, efficacy and toxicity screening studies

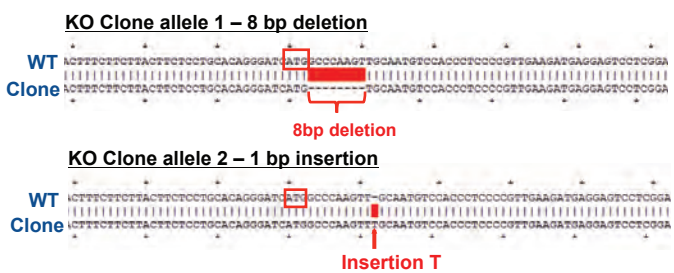
Case Studies

1. Knockout Mutation

Goal: To generate frameshift Knockout mutation in 6 isoforms of a gene in human iPSCs.

How: Six Isoforms of the gene of interest shared the same ATG start codon in the targeted exon. Next generation sequencing (NGS) was used to identify the best gRNA candidate.

Result: Co-transfection of gRNA and Cas9 in the hiPSC resulted in an **8 bp deletion** in allele #1 and a **1 bp insertion** in allele #2 at the Cas9/gRNA cut site, resulting in a frameshift mutation and premature stop codons in all six isoforms of the gene of interest.

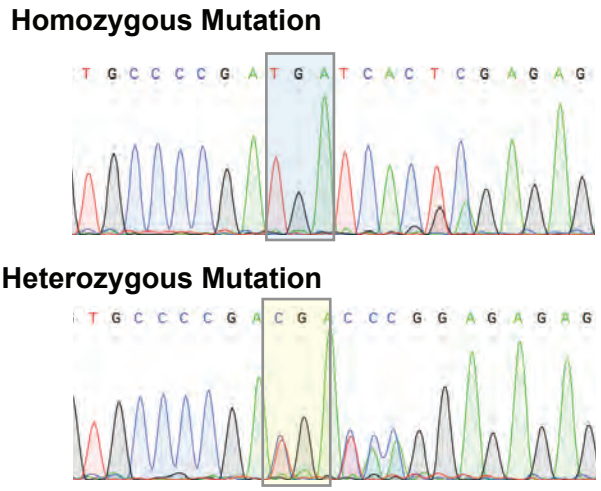


2. Point Mutation Correction in a Patient Cell Line

Goal: To introduce targeted heterozygous and homozygous CGA -> TGA point mutation in the gene of interest in a human iPSC line using CRISPR/Cas9.

How: Two gRNAs, g1 and g2 were chosen after functional validation by NGS, to generate homozygous and heterozygous mutations, respectively, in the gene of interest.

Result: Seven heterozygous clones were identified from 79 total clones screened with g2. One homozygous clone was identified from 76 clones with g1. No off-target activity was observed in the clones after off-target analysis (off-target analysis data not shown).

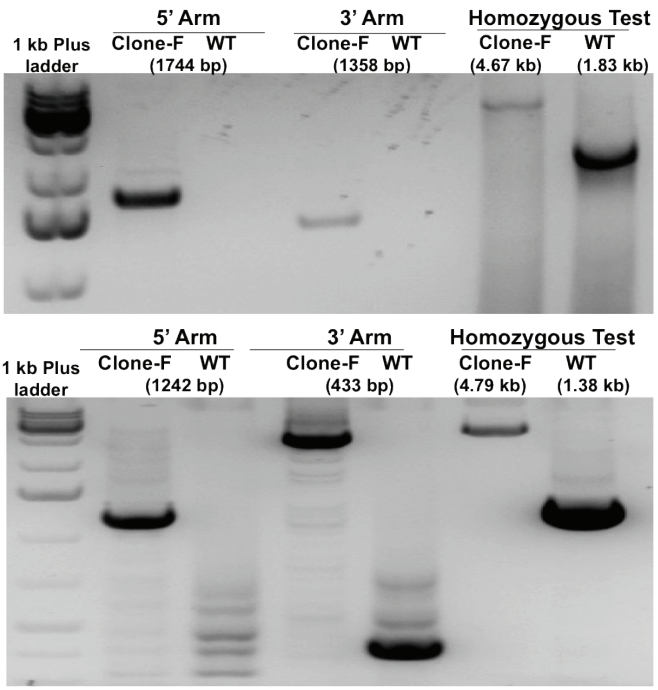


3. Knock-in of Two Reporter Genes in an iPSC Line

Goal: To insert 2 reporter genes, TdTomato and eGFP into ASC's well-characterized control iPSC line (Cat.# ASE-9203) using CRISPR/Cas9.

How: A sequential strategy was adopted to knock-in the 2 reporter genes. First, the TdTomato tag was inserted into the 3' end of the gene of interest #1. A homozygous clone was identified and re-targeted for insertion of the eGFP tag at the 3' end of gene #2. One homozygous clone (Clone-F) was identified and confirmed to have both reporter gene knock-in by PCR and sequencing (not shown).

Result: (Top) PCR gel electrophoresis to confirm the insertion of eGFP into Clone-F at the 3' end of gene #2. (Bottom) PCR gel electrophoresis to re-confirm the presence of TdTomato tag in Clone-F. WT = wild type clone.

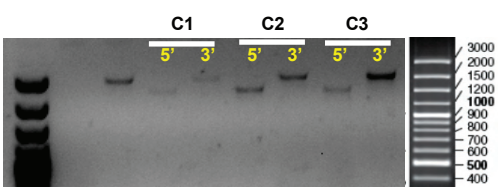


CRISPRCLEAR™

Do-It-Yourself CRISPR Editing Kits for iPSCs

ASC's CRISPR iPSC Gene Editing Kits are designed for your disease- specific studies. This do-it-yourself genome editing toolkit enables generation of CRISPR modified iPSCs in your own lab. Our scientists will work closely with you to design the most efficient CRISPR components to be provided in this custom kit, thereby ensuring a fast turnaround and quality deliverables.

ASK-7020S CRISPR Point Mutation Kit for iPSCs
ASK-7010S CRISPR Knockout Kit for iPSCs
ASK-7030S CRISPR Knock-in Kit for iPSCs
ASK-7040 hH11 Safe Harbor Locus Knock-in Kit
ASK-7042 hAAVS1 Safe Harbor Knock-in Kit



Kit Contains

- Cas9-gRNA plasmid with validation report
- Donor DNA (for knock-in kits)
- Human iPSC line (optional)

Figure: Large transgene knock-in into the hH11 locus in a control iPSC line using the CRISPCLEAR™ Safe Harbor Locus kit. The 5' and 3' junction PCR at the hH11 locus showed a 2.3 kb and a 2.8 kb fragment, confirming transgene insertion at the locus in three clones (C1, C2, C3).

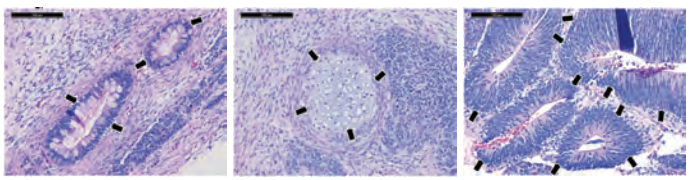
iPSC Characterization Services

Teratoma Formation Analysis Service

Teratoma Analysis is one of the most stringent and accurate quality control assessments for stem cells, in addition to other methods to characterize stemness such as immunohistochemistry and RT-PCR detection of pluripotency markers, in vitro embryoid body formation and karyotyping. It provides a functional assessment of pluripotency of the stem cells, including iPSCs, by analyzing the ability of the cells to form all three embryonic germ cell layers when xenografted into mice. ASC's teratoma formation analysis service has >97% success rate and has been acknowledged in > 30 peer-reviewed publications.

Services includes:

- Cell injection in 2 sites: kidney and testis
- Teratoma harvesting
- Tissue sectioning
- H&E staining
- Histological analysis of teratoma sections



EN: Gland (200x) ME: Cartilage (200x) EC: Neuronal Rosette (200x)

Figure: H&E staining of kidney and testis teratomas from mice injected with the ASE-9203 control iPSC line shows differentiated tissues representing the three germ layers, indicated by arrow heads. EN: endoderm; ME: mesoderm; EC: ectoderm.

Deliverables and Timeline:

- Complete report with histological analysis and high-resolution images of EN, ME, EC formation
- Tissue blocks and H&E stained tissue section slides

Timeline: 1-3 months

Success Rate

Key points	ASC's methods			Traditional methods
Cell Type	mESC/miPSC	hESC	hiPSC	hESC
Teratoma Formation Rate	100%	100%	93.7%	25-40%
Differentiation	distinctive	distinctive	distinctive	poor
Turnaround Time	3-5 weeks	5-8 weeks	10-14 weeks	12-18 weeks
Cells needed	0.5-1 million/site	0.5-2 million/site	1-2 million/site	3-5 million/site

Antibody Pluripotency Marker Staining:
- Human: OCT4, SOX2, SSEA4, TRA-1-60, TRA-1-81
- Mouse: OCT4, SOX2, SSEA1

Pluripotency & lineage-specific marker detection qPCR, RNA-seq

Karyotyping: Chromosome counting; G-banding)

Embryoid Body Formation and Characterization

Germline Transmission Evaluation

mESC Derivation Service

Custom iPSC Culture Service

iPSC Banking Service

Related Services (additional fees may apply)

Cell Immortalization Service
- Patient fibroblasts, primary cells, and more
- Transduction, colony selection (up to 10 passages)
- Characterization (transgene expression by RT-PCR)

Vector Cloning, Virus Packaging Services
- RNAi and inducible vectors/ BAC recombineering
- Retro- and lentivirus packaging service in 10 days (regular, high, ultra-high titers)

iPSC Differentiation Service

ASC's offers iPSC differentiation services for ESC/ iPSC differentiation into more specialized cells including multipotent stem cells and fully differentiated somatic cells. Using proprietary protocols and reagents, we can differentiate your ESCs or patient-derived/ healthy iPSCs into various lineage cells: **neural stem cells (NSC), neurons, astrocytes, cardiomyocytes, hematopoietic lineage cells and hepatocytes**.

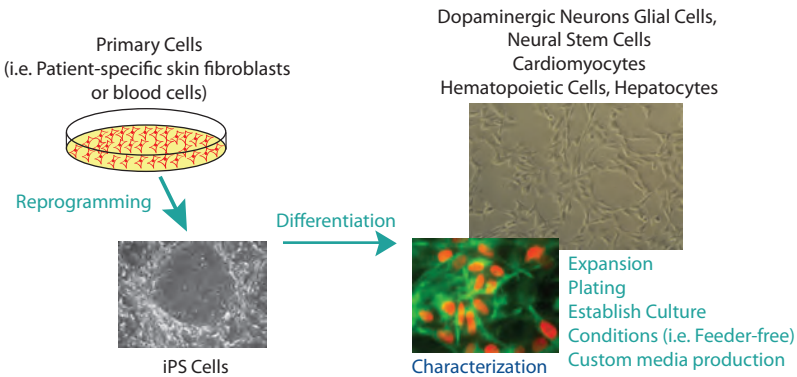
Service includes:

- Recovery and expansion of iPSCs
- Differentiation to precursor cells
- Characterization of precursor cells using immunocytochemical markers and functional assays

Deliverables and Timeline:

- Frozen precursor cells
- Detailed protocols for maturation of precursor cells into lineage of choice (media not included*)
- High-resolution images of immunocytochemical assays
- Detailed milestone and final reports

Timeline: depends on the differentiated lineage



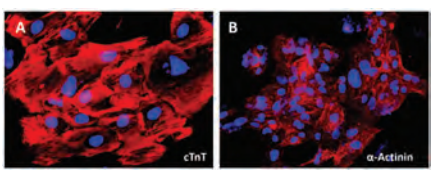
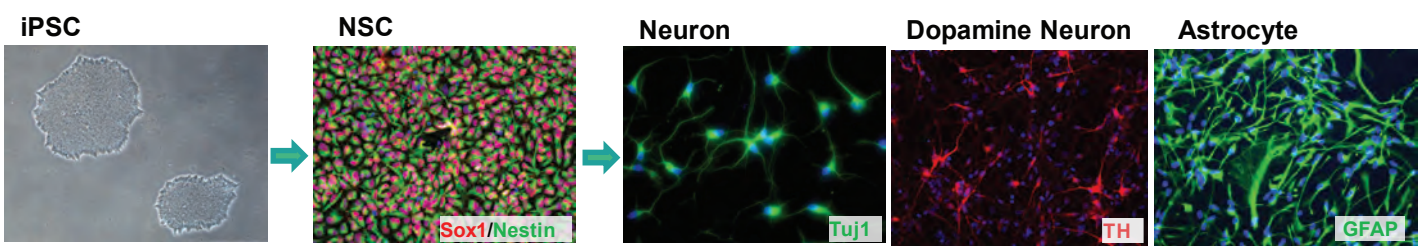


Figure: iPSC-derived cardiomyocytes maintained in cardiomyocyte maintenance medium: >85% of the cells express cTnT and α-actinin.

Neural Stem Cell and Neural Cells Differentiation Service

The process of iPSC differentiation to neurons and neuronal cells is of special importance for neurobiology and related disorders, considering the dearth of clinically relevant *in vitro* models available for research, drug screening and development, and therefore lack of therapy to reverse neuronal damage. ASC's now offers comprehensive service to differentiate your patient-derived iPSCs into self-renewing NSCs or further into neurons (dopaminergic/ cortical) and glial cells, for advancing neuroscience research.



Key Features:

- High purity cells expressing characteristic markers
- Proliferating NSCs can be frozen and cultured for multiple passages while retaining phenotype
- Integration-free differentiation and feeder-free culture protocols
- Functionally viable neurons characterized by immunocytochemistry and functional assays
- Isogenic lineage of NSCs, neurons and glial cells from parental iPSC
- Cells can be grown in co-cultures for generating advanced and bio-relevant cell line models

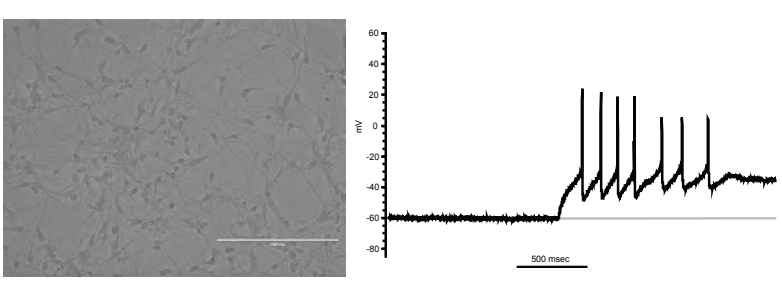


Figure: Single pulse current (patch clamp) recording of dopaminergic neurons derived from iPSCs indicate functional neurons that are excitable upon injection of current.

* Optimized lineage-specific maturation media available separately

Differentiated Neural Stem Cells, Neurons and Astrocytes

Applied StemCell offers high quality NSCs and differentiated neurons and astrocytes derived from fully characterized, parental iPSC lines from multiple donors, for flexibility in choosing the lineage most appropriate for your research.

Key Features:

- High purity, isogenic cells express characteristic cell line markers
- Integration-free protocols
- Fully characterized by immunocytochemistry and whole genome profiling
- Consistent and reliable source of differentiated neuronal cells
- Long-term viability in cell culture
- Neurons and astrocytes can be co-cultured for complex tissue modeling

Benefits and Applications:

- Physiologically relevant tissue models for neurogenesis and CNS function studies
- Drug discovery, neuroprotection and neurotoxicity screening
- Neurodegenerative and neuroinflammation disease modeling
- Co-culture models to study neuronal viability for cell therapy studies

SYMBOL	NSC	NEURONS	ASTROCYTES	DESCRIPTION
AQP4	-23	-5	1082	
CCL2	396	63	2991	
CD44	29	43	8716	
CRYAB	-21	5	9661	
GFAP	-2	1	30956	
HOPX	-14	96	777	
LGALS3	33	8	1203	
NFIA	-43	981	2110	ASTRO
NFIX	-6	720	7849	
PMP2	12	606	3186	
PRRX1	23	19	3973	
S100A6	137	142	1952	
SPARCL1	1092	40	13584	
TNC	164	589	4838	

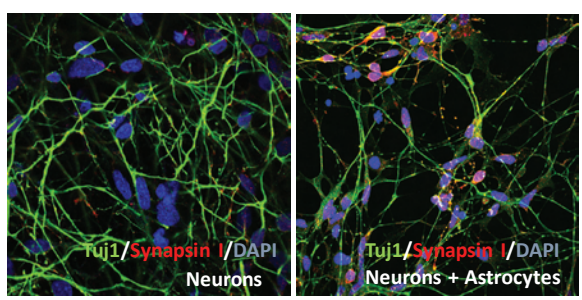


Figure: **(Top right)** Whole genome profiling for markers expressed by differentiated astrocytes. **(Bottom right)** Enhanced synapse formation in neuron-astrocyte co-cultures as seen by increase in synaptic puncta.

Available Control, Patient, Edited iPSCs & Differentiated Lines

Control Human iPSCs:

ASE-9109	Male; cord blood cell-derived; episomal
ASE-9110	Female; cord blood cell-derived; episomal
ASE-9203	Male; fibroblast-derived; episomal
ASE-9101	Male; fibroblast-derived; retrovirus

Differentiated Lines from Control iPSCs:

ASE-9234	NSC; male (from ASE-9109)
ASE-9234F	NSC; female (from ASE-9110)
ASE-9303	NSC; male (from ASE-9203)
ASE-9321	Cortical neurons; male (ASE-9234)
ASE-9321F	Cortical neurons; female (ASE-9234F)
ASE-9322P	Astrocyte Precursor; male (ASE-9234)
ASE-9322PF	Astrocyte Precursor; female (ASE-9234F)
ASE-9322M	Mature Astrocytes; male (ASE-9234)
ASE-9322MF	Mature Astrocytes; female (ASE-9234F)
ASE-9323	Dopamine Neuron; male (ASE-9234)
ASE-9323F	Dopamine Neuron; female (ASE-9234F)
ASE-9027	Cardiomyocytes; PBMC

Patient-derived iPSCs & Differentiated Cells

ASE-9026	NSC; ALS; fibroblasts
ASE-9028	iPSC; Parkinson's Disease; fibroblasts
ASE-9029	iPSC; Parkinson's Disease; Adipose
ASE-9030	iPSC; Parkinson's Disease; PBMC
ASE-9031	iPSC; Diabetes II; fibroblasts
ASE-9033	iPSC; Diabetes II; PBMC
ASE-9040	iPSC; ALS8 P56S; male
ASE-9042	iPSC; ALS8 non-carrier sibling; male
ASE-9043	iPSC; ALS8 P56S; female
ASE-9044	iPSC; ALS8 non-carrier sibling; male
ASE-9045	iPSC; ALS8 P56S; male

Stem Cells (Other Species)

ASE-9005	GermLine mESC C57BL6/129SvJ
ASE-9006	GermLine mESC 129-EZ
ASE-9007	GermLine mESC C57BL/6 EZ
ASE-9008	GermLine mESC BALB/c EZ
ASE-9009	mESC C57BL/6-ALBINO
ASE-9106	Pig iPSCs
ASE-9107	Mouse iPSCs
ASE-9108	Guinea Pig iPSC**s**

Neurological Disease KO iPSC lines: (from ASE-9109)

ASE-9400	PARK2-/-; Parkinson's disease
ASE-9401	PARK7-/-; Parkinson's disease
ASE-9402	PINK1-/-; Parkinson's disease
ASE-9403	LRRK2-/-; Parkinson's disease
ASE-9404	BDNF-/-; CNS
ASE-9405	APOE-/-; Alzheimer's disease
ASE-9406	DISC1-/-; Schizophrenia
ASE-9407	SOD1-/-; ALS
ASE-9408	CNTNAP2-/-; Autism

Neuronal Knock-in Reporter Lines: (from ASE-9109)

ASE-9500	MAP2-Nanoluc/Halotag
ASE-9501	GFAP-Nanoluc/Halotag
ASE-9502	AAVS1-DCXp-GFP

Safe Harbor Locus Reporter Lines: (from ASE-9109)

ASE-9503	CAG-GFP-Chr19
ASE-9504	CAG-GFP-chr13

Neurotoxicity Drug Screening Service

iPSC-based drug screening is the future of therapeutic drug development and recognized by international drug regulatory agencies. As one of the leaders in the stem cell industry, Applied StemCell (ASC) can help drug developers prioritize and move promising therapeutic compounds to the next stage of FDA approval faster and efficiently. We offer a comprehensive cell-based test battery for drug target discovery, drug efficacy and neurotoxicity screening.

Generation of iPSCs lines
- Control iPSCs
- Engineered iPSCs to model disease
- Engineered reporter lines
- CRISPR Mutation Correction

Differentiation to Neural Cells
- Neural stem cells (NSC)
- Neurons (dopaminergic, cortical)
- Astrocytes
- Oligodendrocytes

Disease Modeling & Drug Screening
- Neurotoxicology assays
- Neuroprotection screening
- CNS drug efficacy testing
- Screening for new drug targets

Key Features:

- Ethically-compatible, physiologically relevant models
- Fast & cost-effective screening
- Regulatory-compliant
- Highly predictive models with reproducible, consistent quality of results
- Stage-specific phenotype screening in a variety of tissues from different sources
- Ready-to-use or custom generated panels of iPSCs & derived cells

Benefits & Applications:

- Improved drug discovery
- Reliable safety assessments
- Prioritize your drug candidates
- Reduce late-stage drug attrition

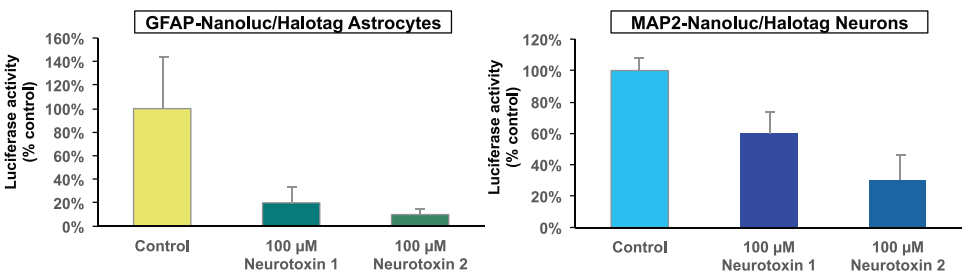


Figure: Luciferase-based cell viability was significantly reduced by up to 90% in astrocytes and neurons derived from lineage-specific reporter iPSC lines, when exposed to two neurotoxins. Luciferase activity was measured as % of control (DMSO-treated cells).

Table. iPSC-derived dopamine neurons provide predictive & reliable results in neuroprotective assays. Out of 40 compounds that were neuroprotective in conventional cell based assays, only 18 compounds (used in human clinical trials) were found to be neuroprotective in an MPP+- iPSC-derived dopamine neuron model of Parkinson's disease.

Neurotransmitter/ MAO Inhibitors:	Rasagiline, selegiline, nicotine, topiramate, amantadine, zonisamide, taurine
Antioxidant/ Mitochondrial Stabilizers:	Resveratrol, N-acetyl cysteine, lipoic acid, epigallocatechin gallate, creatine
Anti-Inflammatories:	Rolipram, indomethacin, 7-nitroindazole, 3-aminobenzamide, phenanthridone

Comprehensive test battery measures multiple morphological and physiological endpoints

Cellular morphology and biomarker screening
Cytotoxicity and cell viability assays
Mitochondrial toxicity testing
Functional assays (electrophysiology)
Quantitative gene expression: qPCR and RNA-seq (NGS)
Custom assay development to accommodate specialized needs of customers and drug candidates

iPSC Cell Culture Products

MEF Cells

- Strict quality control tested for long term culture of either mouse or human ESC/iPSC lines (>168 passages)
- Our MEFs are used by more than 200 clients in academic and industrial labs worldwide
- DR4, CF-1, Neo, SNL 76/7 cells available (Untreated, irradiated, mitomycin C-treated)

We provide high-quality MEF cells!
Average viability >95%

Cell Culture Products

- Serum and feeder free medium
- Conditioned medium for human ESC/iPSC culture
- Freezing medium

Easy-to-use Kits

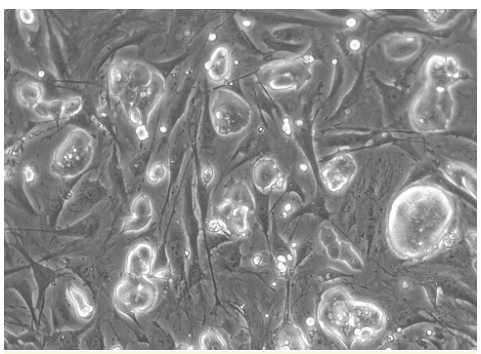
- EZ iPSC generation kit (Episomal/Retroviral)
- iPSC characterization kit
- Immortalization kit

Primary Cells

- Fibroblasts (Healthy/Disease)
- Cardiomyocytes, Cardiac Progenitor Cells

ESC-Sure™ FBS

- High quality, affordable ESC-grade serum for ES and iPS cell culture
- Supports undifferentiated growth of mESCs (QC report provided)



ESC-Sure™ FBS is extensively tested for supporting undifferentiated growth of mouse ESCs (mouse embryonic stem cells). The image shows healthy mESC that were cultures in MEF-feeder cell dependent system along with ASC's ES-grade FBS.

My EZGel™ iPSC 3D Matrix

- Serum-free and feeder-free cell culture
- Neutral pH and room temperature culture/ 37°C: No more icing or acidic conditions
- Mimics cell microenvironment
- Cells are easily encapsulated and harvested
- Fast hydrogel formation (~30 min) and longer shelf life
- Supports the formation of the germ layers (teratoma formation)

iPSC and Differentiated Cell Culture Medium and Related Products

ASE-9234SM	Neural Stem Cell Maintenance Media	ASE-9323K	Dopa Neuron Starter Kit (from male, cord blood iPSCs)
ASE-9321K	Neurons Starter Kit (from male, cord blood iPSCs)	ASE-9323KF	Dopa Neuron Starter Kit (from female, cord blood iPSCs)
ASE-9321KF	Neurons Starter Kit (from female, cord blood iPSCs)	ASE-9323DI	Dopa Neuron Induction Media
ASE-9321NI	Neuron Induction Media	ASE-9323DM	Dopa Neuron Maintenance Media
ASE-9321NM	Neurons Maintenance Media	ASM-4013	NeuroSure™ NSC Differentiation Media
ASE-9322K	Astrocytes Starter Kit (from male, cord blood iPSCs)	ASM-4014	NeuroSure™ NSC Maintenance Media
ASE-9322KF	Astrocytes Starter Kit (from female, cord blood iPSCs)	ASM-5008	ESC-Sure™ Conditioned Medium for Human ESC/iPSC Culture
ASE-9322AI	Astrocytes Induction Media	ASM-5010	ESC-Sure™ Serum-/Feeder- Free hESC/iPSC Culture Medium (SFFM)
ASE-9322AM	Astrocytes Maintenance Media	ASM-5011	ESC-Sure™ Mouse ESC Mate for mESC/iPSC culture

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