

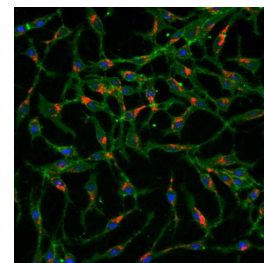
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Version 2

🌐 LUHMES (lund human mesencephalic) culturing and differentiation protocol V.2

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Manuscript citation:

ATCC <https://www.atcc.org/products/crl-2927#product-references>

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Protocol status: Working

We use this protocol and it's working

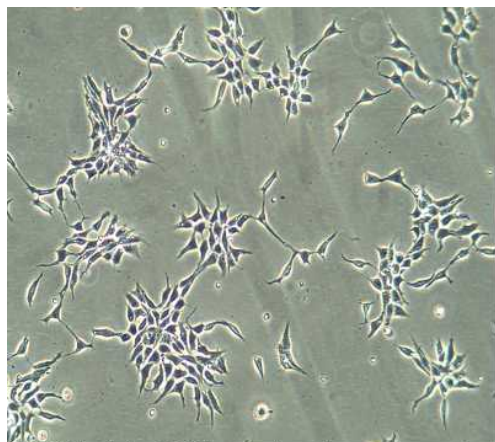
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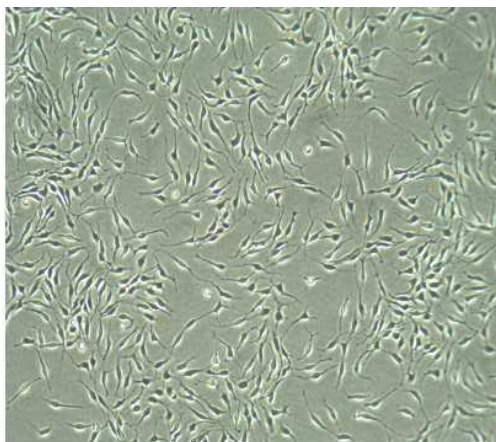
Protocol Integer ID: 95260

Keywords: human mesencephalic, lund human mesencephalic, luhme, differentiation protocol

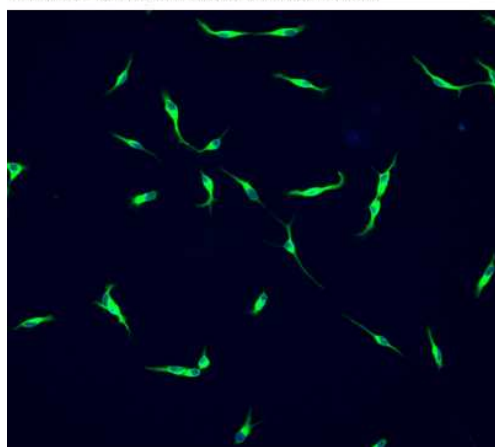
Abstract



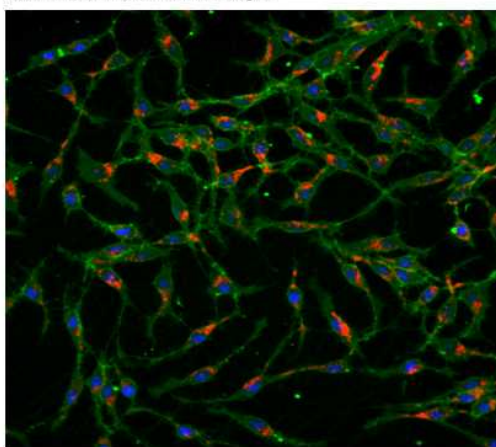
LUHMES at about 50% percent confluency



LUHMES Differentiation Day 2



LUHMES Differentiation day 4 with MAP2/rabbit Ab



LUHMES Differentiation day 2. Stained with Cell mask orange, lyso-tracker deep red and Hoechst

Materials

	A	B	C	D
	Reagents	Stock Concentration	Final Concentration	Final Solution Volume = 20mL
	DMEM/F12	x	x	19,792uL
	B27	50X	1X	200ul
	Recombinant Human FGF basic (100ug) store at -20C	100ug/mL Reconstitute: add 1000ul PBS to 100ug vial of fgf	40ng/mL	8uL
	Penicillin Streptomycin (10,000 U/mL)	X	X	20uL

LUHMES Growth Media

	Reagents	Note	Stock Concentration	Working Concentration	Final Solution Volume = 20mL
	DMEM/F12		X	X	19,568uL
	B27-supplement		100X	1X	200uL
	DibutyrilcAMP MW: 491.4 g/mol Mass: 100mg	Reconstitute: Add 1.56mL of PBS to 100mg Vial	100mM	1mM	200uL
	Ascorbic Acid (500mg Vial)	Reconstitute: Add 14.195mL PBS (pH 7.2) to 500mg of ascorbic acid	200mM	0.2mM	20uL



	Reagents	Note	Stock Concentration	Working Concentration	Final Solution Volume = 20mL
	Human Recombinant LIF (50ug vial)	Reconstitute: Add 500ul of nuclease free water to 50ug vial. *2 weeks or at -20°C to -80°C for up to 3 months	0.1ug/mL	10ng/mL	2uL
	Human Recombinant BDNF (10ug vial)	Reconstitute: Add 100ul to vial. centrifuge d before opening, Do Not Vortex	0.1 mg/mL	20ng/mL	4uL
	Human Recombinant GDNF (10ug vial)	Reconstitute: Add 100ul of nuclease free water to 10ug vial of GDNF. Make 5ul aliquots Store aliquots at -20°C	0.1mg/mL	20ng/mL	4uL
	Tetracycline MW: 480.91 g/mol Mass: 500mg	Reconstitute: Add 50mL of bio-grade water to 500mg of tetracycline. Store in -20	10mg/mL	1ug/mL	2uL
	TGF β-III (10ug vial)	Storage Conditions 4° C	0.25 mg/mL	20ng/mL	0.25 mg/mL

LUHMES Differentiation Media



✕ Tgf beta 3 (human) Recombinant Protein **Invitrogen - Thermo Fisher Catalog #RP8600**

✕ Human Recombinant LIF **STEMCELL Technologies Inc. Catalog #78055**

✕ Dibutyl-cAMP **STEMCELL Technologies Inc. Catalog #73884**

✕ Tetracycline Hydrochloride **Thermo Scientific Catalog #A39246**

✕ Human Recombinant GDNF **STEMCELL Technologies Inc. Catalog #78058**

✕ Human Recombinant BDNF **STEMCELL Technologies Inc. Catalog #78005**

✕ Absorbic Acid **STEMCELL Technologies Inc. Catalog #72132**

Protocol materials

✕ Poly-L- Ornithine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A-004-C**

✕ Fibronectin human plasma,liquid, 0.1% (Solution), **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F0895-1MG**

✕ Penicillin Streptomycin (10,000 U/mL) **Gibco - Thermo Fisher Scientific Catalog #15140122**

✕ B-27 Supplement (50X) **Thermo Fisher Scientific Catalog #17504044**

✕ Recombinant Human FGF basic/FGF2/bFGF (145 aa) Protein, CF **R&D Systems Catalog #3718-FB**

✕ DMEM/F12 **Thermo Fisher Scientific Catalog #11320033**

✕ Trypsin/edta Solution (TE) **Thermo Scientific Catalog #R001100**

✕ Human Recombinant BDNF **STEMCELL Technologies Inc. Catalog #78005**

✕ Human Recombinant LIF **STEMCELL Technologies Inc. Catalog #78055**

✕ Dibutyl-cAMP **STEMCELL Technologies Inc. Catalog #73884**

✕ Tetracycline Hydrochloride **Thermo Scientific Catalog #A39246**

✕ Human Recombinant GDNF **STEMCELL Technologies Inc. Catalog #78058**

✕ Tgf beta 3 (human) Recombinant Protein **Invitrogen - Thermo Fisher Catalog #RP8600**

✕ Absorbic Acid **STEMCELL Technologies Inc. Catalog #72132**



LUHMES coating protocol

- 1  Poly-L- Ornithine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A-004-C**
 - [M] 0.1 mg/mL Stock concentration
 - [M] 50 µg/µL Working concentrationThaw an aliquot of Poly-L-Ornithine solution at room temperature.
- 2 Dilute Poly-L-Ornithine solution to 50ug/mL in Nuclease-free water

Add  500 µL of PLO for every  500 µL Nuclease-free water
- 3 Add 7mL of the 50ug/mL PLO to a T-75 overnight at RT.
- 4 Rinse flask 3 times with bio-grade water.
- 5 Allow the flask to air dry for 15 minutes uncapped and standing upright in the hood. (turn on UV)
- 6  Fibronectin human plasma,liquid, 0.1% (Solution), **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F0895-1MG**
Size: 100uL, Storage Temperature: -20 °C in aliquots
 - [M] 1 mg/mL Stock Concentration
 - [M] 2 µg/µL Working concentrationThaw an aliquot of Poly-L-Ornithine solution at room temperature.

Note

Do not vortex or shake vigorously to resuspend the fibronectin. This will cause the fibronectin to “crash” out of solution, which is irreversible
- 7 Dilute the Fibronectin in sterile Hank’s Balanced Salt Solution (HBSS).

Add  2 µL Fibronectin to  998 µL HBSS
- 8 Place fibronectin coated flask in incubator for 3 hours



- 9 Rinse 3 times with HBSS.
- 10 Air dry for 15 minutes and add LUHMES growth media.

LUHMES growth media

- 11 Change (pre-warmed) media every 1-2 days

Note

LUHMES are sensitive to changes in the media pH and oxidative stress. Always use fresh DMEM/F12 because the HEPES buffer in DMEM is subject to photooxidation upon exposure to light and produces hydrogen peroxide.

- 12  DMEM/F12 **Thermo Fisher Scientific Catalog #11320033**

 Penicillin Streptomycin (10,000 U/mL) **Gibco - Thermo Fisher Scientific Catalog #15140122**

 B-27 Supplement (50X) **Thermo Fisher Scientific Catalog #17504044**

 Recombinant Human FGF basic/FGF2/bFGF (145 aa) Protein, CF **R&D Systems Catalog #3718-FB**

- Add recombinant human FGF to media after seeding cells

LUHMES Passaging (~ every 2-3 days)

- 13 Remove media and rinse with DPBS
- 14 Add fresh culture media to flask for at least 15 minutes before seeding cells to allow media to reach normal pH
- 15 Add 4mL of the pre-warmed .025% Trypsin/EDTA and place in the incubator for 3 minutes.  Trypsin/edta Solution (TE) **Thermo Scientific Catalog #R001100**
- 16 Neutralize trypsin with 6mL of pre-warmed DMEM/F12





- 17 Transfer cells to 15mL tube and centrifuge for 5 minutes at 1200 RPM
- 18 Discard the supernatant and resuspend in 1mL LUHMES growth media
- 19 use a 5mL pipette to Triturate cells only 1 or 2 times before seeding.

Cryopreservation

- 20 Label 2mL cryovials with the date, name, FIV#, Qbench number, passage number and cell type.

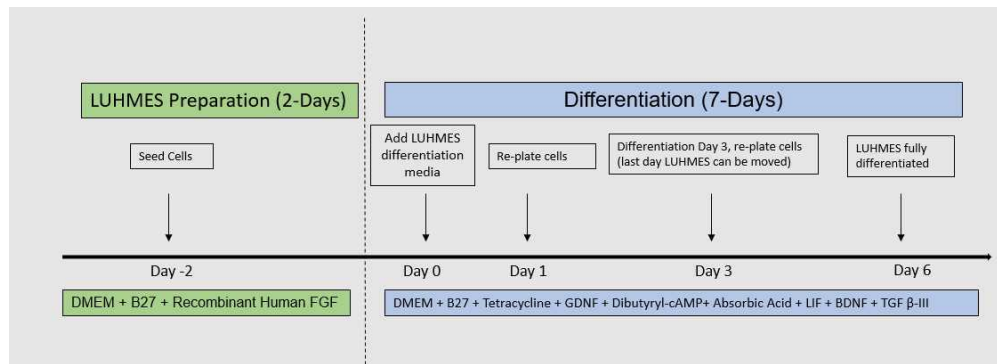
Add about 1.5 million cells per vial with 1mL freezing media.

Freezing Media:

- 7mL LUHMES Media
- 4uL B-fgf (40ng)
- 2mL FBS (20%)
- 1mL DMSO (1%)

LUHMES Differentiation

- 21 Day 0, when the LUHMES are 80% confluent, add differentiation media and incubate overnight.
- 22 Day 1, replate cells onto a new poly-l-ornithine and fibronectin-coated plate. Replate cells at a density of 1 million cells per T-75 flask or ~ 400,000 per well of a 6-well.
- 23 Replace LUHMES differentiation media every day while differentiation (neurons become mature in 7-days)



LUHMES Differentiation Timeline

Protocol references

ATCC LUHMES CRL-2927