

Neural Crest differentiation from hPSCs

On Biolaminin® 521

Biolaminin 521 is a full-length, human, recombinant laminin 521 cell culture substrate. It provides an optimal environment for feeder-free culture of human PSCs under chemically defined, animal origin-free conditions. Biolaminin 521 can support Neural Crest (NC) induction from hPSCs using STEMdiff™ Neural Crest Differentiation Kit (STEMCELL Technologies) to establish a fully adherent protocol and re-place undefined coating material. With this protocol hPSC-derived neural crest cells can be established under serum-free and defined condition using the STEMdiff™ Neural Crest Differentiation kit and Biolaminin 521. hPSCs that are maintained on Biolaminin 521 do not need further adaption for this application.

For detailed coating instruction, follow [Instruction for use 001](#).



For detailed instruction of hPSC culture on Biolaminin 521, follow [Instruction for use 003](#)



Protocol

- 1 Coat culture ware with 5µg/mL LN521 at 4°C overnight.**

Replace coating solution with 0.2ml/cm² complete NC medium (+ ROCK inhibitor) and prewarm to room temperature before cell seeding. Do not let the coated surface dry to avoid inactivation of the coated LN521.
- 2 Complete the NC medium and follow the commercial STEMdiff Neural Crest Differentiation Kit (STEMCELL Technologies) instruction.**
- 3 Passage cells at their proliferation stage by lifting cells from 70-99% confluent plate.**
 - Wash cells with 1x DPBS-/-.
 - Add 0.2-0.5ml/cm² enzyme or non-enzyme reagent, here follow the NC kit instruction, Gentle Cell Dissociation Reagent to culture ware, incubate at 37°C for 5-10min.
 - Add equal volume of DMEM/F12 or medium to the well and pipet 1- 4 times to achieve single cell suspension.
 - Centrifuge cells at 100-300g for 5 min. Remove supernatant and resuspend cells in completed NC medium + 10µM Rocki.
- 4 Perform a complete medium change without Rocki and feed cells daily.**

(Regular maintenance of human pluripotent stem cells (hPSC) on Biolaminin 521 does not require ROCK inhibitor)
- 5 Neural crest cells are ready from 5-7 days for analysis or further differentiation (to be explored).**

Neural crest cells can be assessed by IF staining of NC markers such as CD271 or SOX10. Depending on cell lines, there might be population of PAX6 positive cells in the yield cells.

[Learn more on the Neural Crest Appnote](#)

