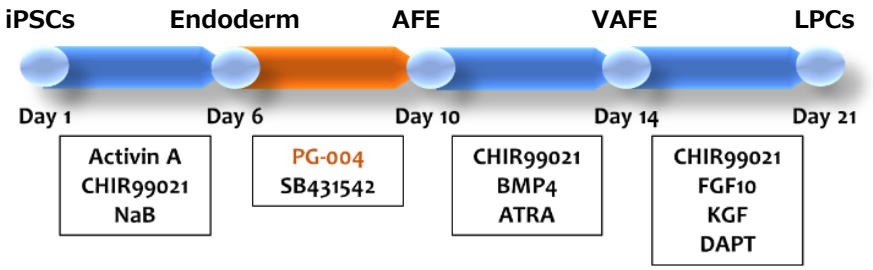
PeptiGrowth

Application Note:
PG-004 Noggin-like peptide

<Induction of lung progenitor cells from hiPSCs and formation of lung alveolar organoids>

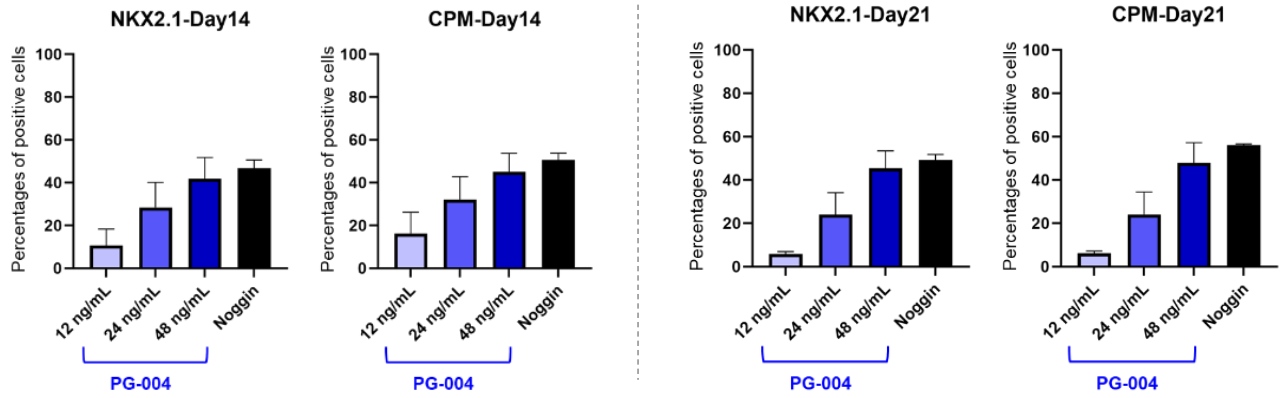
Summary: PeptiGrowth Inc. has developed a novel synthetic peptide possessing equivalent activity to Noggin (product code: PG-004), which is an antagonist of Bone Morphogenetic Protein (BMP). The product was launched for sale in June 2022. Using this product, the induction of lung progenitor cells (LPCs) from hiPSCs and the formation of lung alveolar organoids by these cells were demonstrated.

Outline: When generating LPCs from hiPSCs, Noggin is traditionally used in the induction stage of anterior foregut endoderm (AFE) from endoderm. By comparing the efficiencies of differentiation of ventralized AFE(VAFE) and LPCs using either Noggin or PG-004, we observed a concentration-dependent increase in the induction efficiency of each cell type with PG-004. At a concentration of 48 ng/mL of PG-004, the results were comparable to those achieved with Noggin. Additionally, we confirmed the formation of lung alveolar organoids using LPCs generated through the differentiation process with PG-004.



No.	Additive Factor	Concentration (ng/mL)
1	Noggin	100
2	PG-004	12
3		24
4		48

Differentiation induction process and added factors from iPSCs to LPCs.
After maintaining and expanding human iPSCs (HILC01 strain) in commercially available culture medium (mTeSR Plus-cGMP), a differentiation induction was conducted. (Ref : *Nature Methods*. 2017, **14**(11): 1097-1106)
(1) Culturing iPSCs in media with Activin A, CHIR99021 and sodium butyrate (NaB) for 6 days to generate endoderm.
(2) Generating AFE by culturing endoderm in media with SB4315432 and either Noggin or PG-004 for 4 days.
(3) Treating AFE with CHIR99021, BMP4 and all-trans-retinoic acid(ATRA) for 4 days to generate VAFE.
(4) Culturing VAFE in media containing CHIR99021, FGF10, KGF and DAPT(γ-secretase inhibitor) for 7 days to induce differentiation into LPCs.



Differentiation efficiency of VAFE[Day 14](above left figure) and LPCs[Day 21](above right figure)
The differentiation markers NKX2.1 (Homeobox protein Nkx-2.1, an early transcription factor in lung cells) and CPM (Carboxypeptidase M) were analyzed by flow cytometry using specific antibodies, and the positive cell ratios (%) in the entire cell population were determined. In both VAFE and LPCs, the positive ratios for NKX2.1 and CPM increased with the concentration of PG-004. In the group treated with 48 ng/mL PG-004, the results were comparable to the group treated with Noggin at a concentration of 100 ng/mL. Each graph represents the average value of three independent experiments with standard errors [Continued on the next page].

(Data provided by : HiLung Inc.)

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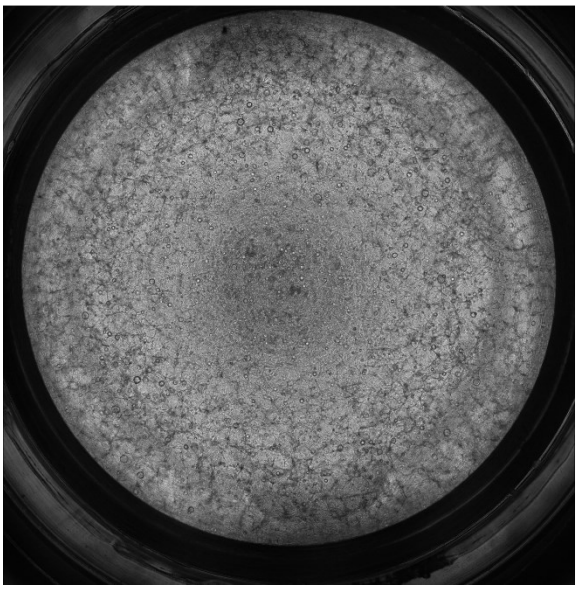
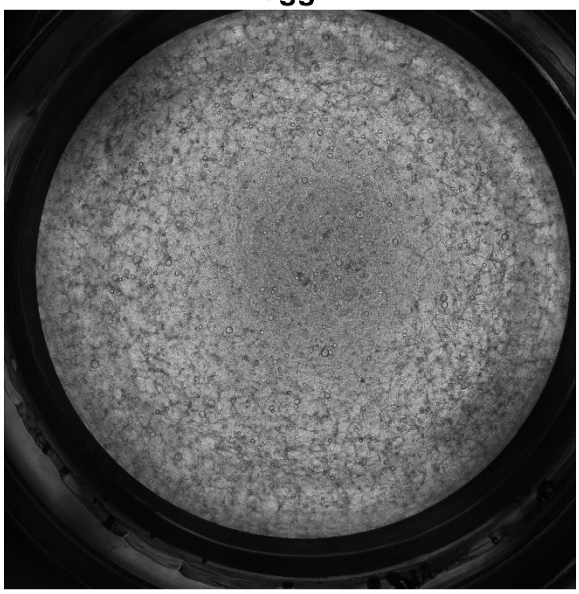
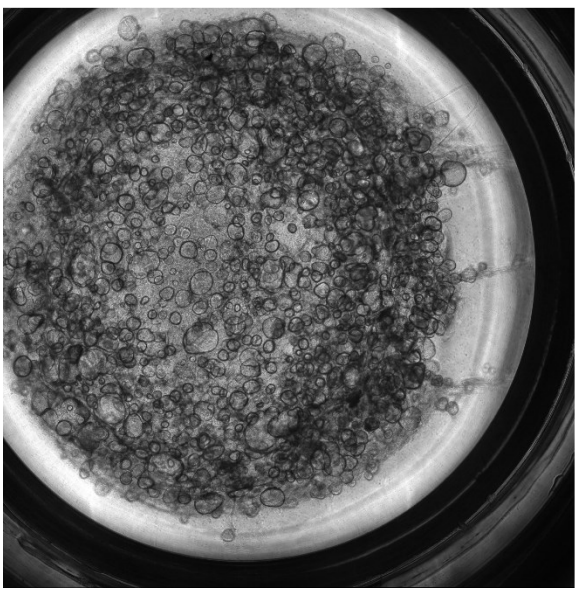
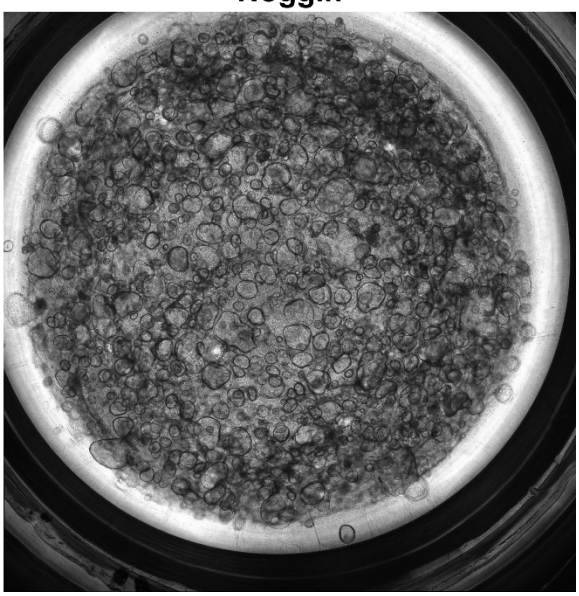
Application Note:

PG-004 Noggin-like peptide

<Formation of Lung Alveolar Organoids Using LPCs induced by PG-004>

	PG-004	Noggin
Day 6		
Day 14		

Lung alveolar organoids formed using LPCs (Day 6: upper images, Day 14; lower images)
Following the procedure outlined on the previous page, PG-004 was used at a concentration of 48 ng/mL to induce differentiation from iPSCs to FAE and ultimately to LPCs. Isolation and purification of LPCs were performed using the specific surface antigen CPM. Lung organoids were then formed from these isolated and purified LPCs. The morphology and other characteristics were found to be comparable to those induced from LPCs under the influence of recombinant Noggin.

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