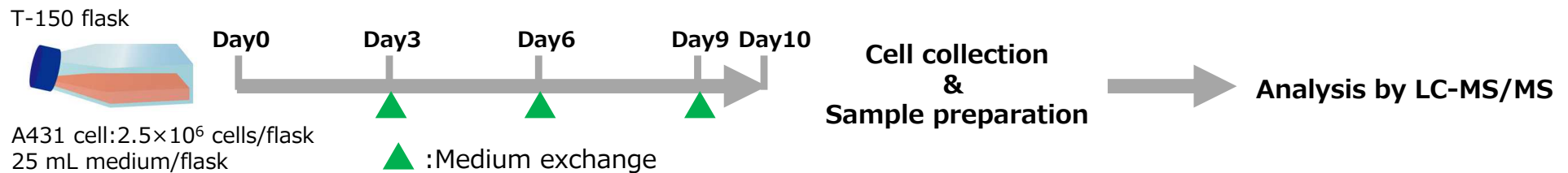


## Conclusion :

After culturing for **10 days** with either **10 ng/mL** or **1000 ng/mL** of PG-001, the residual amount of PG-001 within the cells was **below the detection limit** for both conditions.  
→ **PG-001 does not remain in the final products.**

## Procedure of residual property test for PG-001



### Medium Composition

- DMEM, High Glucose, GlutaMAX, Pyruvate (Thermo)
- 10 % FBS (Thermo)
- 1X MEM NEAA Solution, 100X (Gibco)
- 1:1000 Gentamicin, 50 mg/ml (Nacalai)
- 0.01% DMSO
- **PG-001: 10 ng/mL (approx. 2.07 nM) or 1000 ng/mL (approx. 207 nM)**

### Sample Preparation & Analysis

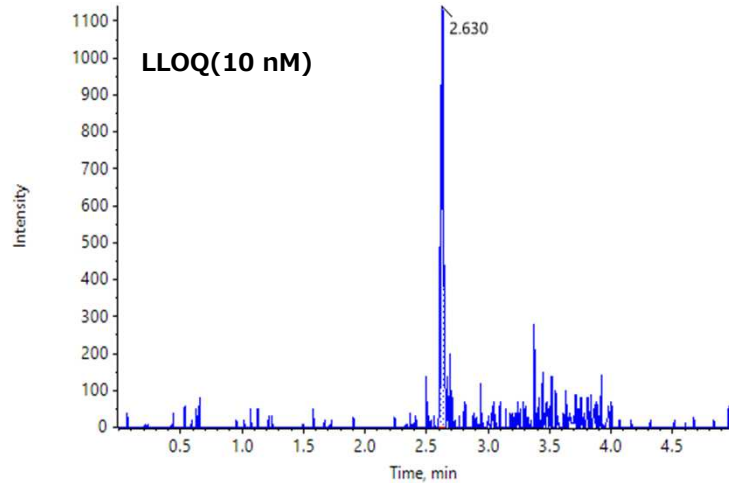
- 1) Dispense into  $1.0 \times 10^6$  cells/tube
- 2) Cell lysis by freeze & thaw, twice
- 3) Addition of extraction solvent (3%, formic acid/ACN, 20 nM Veparamil)  
→ **Internal Standard**
- 4) Centrifugation at 15,000xg, 10 min at 4°C
- 5) Take the supernatant and analysis by LC-MS/MS.

### Calibration standard preparation

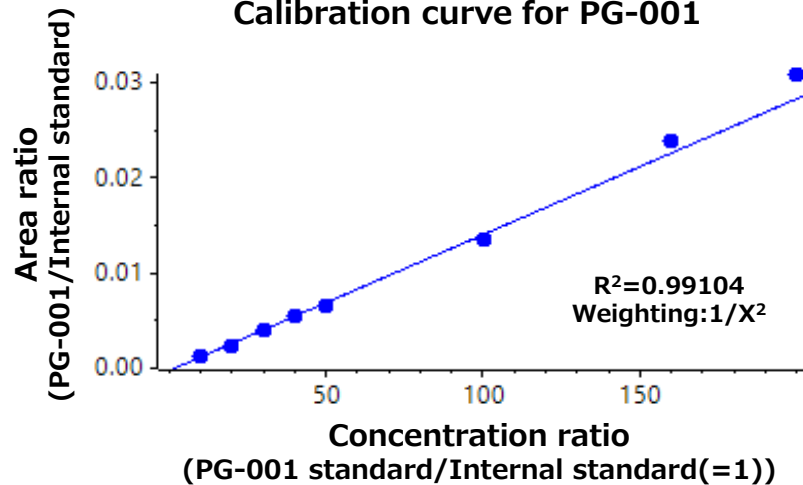
- 1) A431 cell pellet (cultured without PG-001)
- 2) Dispense into  $1.0 \times 10^6$  cells/tube
- 3) Cell lysis by freeze & thaw, twice
- 4) Addition of 50 uL, extraction solvent with different conc. of PG-001 **0(Blank), 10, 20, 30, 40, 50, 100, 160, and 200 nM**
- 5) Centrifugation at 15,000xg, 10 min at 4°C
- 6) Take the supernatant and analysis by LC-MS/MS

## Result detail

Example of chromatograms of PG-001



Calibration curve for PG-001



**Lower limit of Quantification(LLOQ):**  
**10 nM → 2.35 ng** (10 nM × 4705.29 g/mol × 50 uL)

## Measurement result of LC-MS/MS

10 ng/mL

Area:PG-001	Area: IS	Observed Conc.(nM)
N/A	1335384	Below LLOQ
91	1347377	Below LLOQ
79	1349176	Below LLOQ

1000 ng/mL

Area:PG-001	Area: IS	Observed Conc.(nM)
54	1352723	Below LLOQ
44	1359470	Below LLOQ
54	1353460	Below LLOQ

Calibration standard:10 nM(LLOQ)

Area:PG-001	Area: IS	Observed Conc.(nM)
1689	1310059	10.7 nM

**✓ PG-001 does NOT remain in cells**