

**PRODUCT: U87MG VIII 4.12**

**CATALOG NUMBER: CL 01004-CLTH**

**SHIPPED IN:** dry ice

**STORAGE:**

Note: For best results begin culture of cells immediately upon receipt. If this is not possible, store at -80°C until first culture. Store subsequent cultured cells long term in liquid nitrogen.

**QUANTITY & CONCENTRATION:**

1 mL, 1 x 10<sup>6</sup> cells/mL in 80% MEM, 10% FBS, 10% DMSO

**PHYSICAL FORM**

U87MG VIII 4.12 clone cell lines are provided to customers in vials containing >1x10<sup>6</sup> cells/mL

**BACKGROUND/DESCRIPTION**

The U87MG VIII 4.12 cell line is a permanent line established from cell line derived from human malignant glioma, classified as glioblastoma. U87MG VIII (4.12) cell line stably expresses high levels of EGF mutant receptor VIII and G418-resistance gene introduced by plasmid's stable transfection.

**QUALITY CONTROL**

The cryovial contains at least 1.0 × 10<sup>6</sup> U87MG VIII (4.12) cells as determined by morphology, antibiotic selection and viable cell count. U87MG VIII (4.12) cells are tested free of microbial contamination.

**MEDIUM**

Complete Growth Medium: the base medium for this cell line is: MEM.

To make the complete growth medium, add the following components to the base medium:  
10% fetal bovine serum (FBS), 1% Pen-Strep, 0,2% Gentamycin, (optional) 200 µg/mL G418.

Freeze Medium: 80% MEM, 10% FBS, 10% DMSO.

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## UNPACKING & STORAGE INSTRUCTIONS

1. Check all containers for leakage or breakage.
2. Thaw the frozen cryovial according to subculturing procedure.
3. Optimally: Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below 80°C, preferably in liquid nitrogen vapor, until ready for use.

## HANDLING PROCEDURE FOR FROZEN CELLS

### Establishing U87MG vIII (4.12) Cultures from Frozen Cells

1. Place 10 mL of complete MEM growth medium in a 50-mL conical tube and warm to room temperature.
2. Thaw the frozen cryovial of cells by gentle agitation in a 37°C water bath. Decontaminate the cryovial by wiping the surface of the vial with 70% (v/v) ethanol.
3. Pipet cells gently into a sterile 15ml conical tube and slowly add 5ml complete MEM growth medium dropwise to conical tube containing cells.
4. Collect the cells by centrifugation at 1000 rpm for 5 minutes at room temperature. Remove the growth medium by aspiration.
5. Resuspend the cells in the conical tube in 15 mL of fresh growth medium by gently pipetting up and down.
6. Transfer the 15 mL of cell suspension to a T-75 tissue culture flask. Place the cells in a 37°C incubator at 5% CO<sub>2</sub>.
7. Monitor cell density daily. Cells should be passaged when the culture reaches 95% confluence.

Medium Renewal: Thrice per week.

## SUBCULTURING PROCEDURE

1. Discard culture medium.
2. Briefly rinse the cell layer with PBS and discard it.
3. Add 1 mL 0.05% (w/v) Trypsin - 0.53 mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 minutes). To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Centrifuge cells 200xg for 5 min and suspend cells in fresh Complete Growth Medium.
6. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37°C, 5% CO<sub>2</sub>

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### SAFETY PRECAUTION

AMS Biotechnology Europe recommends using protective gloves and clothing and wearing a full face mask always when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. During thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

The product should be handled by trained personnel observing good laboratory practices. It is important to avoid breathing vapor, avoid skin contact or swallowing.

### BIOSAFETY LEVEL: 1

Appropriate safety procedures should always be used with this material. Please check all safety procedures required in your country.

### WASTE DISPOSAL

It is highly recommended that waste always be returned to special company responsible for utilizing such type of waste.

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