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## Dulbecco's Modified Eagle's Medium / Ham's Nutrient Mixture F12 (DMEM /F12)

w/ L-Glutamine w/ 15mM HEPES w/o Sodium Bicarbonate

CAT  $N^\circ$  : SPD-102-1L

**Theoretical pH** :  $5.8 \pm 0.3$ 

**Osmolality** : 279 mOsm/kg  $\pm$  10%

**Colour** : off-white powder

**Storage conditions** : Store dry powder medium at  $+2^{\circ}$ C to  $+8^{\circ}$ C

Store hydrated medium at  $+2^{\circ}$ C to  $+8^{\circ}$ C, protected from light

Shelf life : 36 months

**Endotoxin** : < 1 EU/ml

Composition : Displayed on website and in catalogue; also available on request.

## **Recommended use :**

- Respect storage conditions of the product

- Do not use the product after its expiry date

- Store the product in a dry area

- Wear clothes adapted to the manipulation of the product to avoid contamination (e.g. : gloves, mask, hygiene cap, overall...)

- Protect the product from any form of humidity

- Use, in one time, after opening, the entire quantity of product of the container, without making a concentrated solution (to avoid the formation of precipitates). If it is not possible, close the container immediately after sampling the quantity of powder required.

- Supplements can be added prior to sterile filtration of the medium or aseptically introduced to sterile medium (respect the final concentration of the media). The nature of the supplements may affect storage conditions and shelf life of the medium.

The product is intended to be used in vitro, in laboratory only. Do not use it in therapy, human or veterinary applications.

## **Application** :

DMEM-F12 contains 15mM HEPES to provide additional buffering capacity to the medium. A zwitterionic buffer, HEPES has a pKa of 7.3 at 37°C, which is more compatible with most culture systems than the pKa of sodium bicarbonate which is 6.2 under similar conditions. HEPES will reduce sudden, drastic pH shifts, but as with other buffers, it will not prevent pH shifts entirely.

## **Preparation instructions :**

- 1) Measure 80 90% of final required volume of water. Water temperature should be 15-30°C.
- 2) While gently stirring the water, add slowly the powdered medium (15.601 g/litre). Stir until dissolved. Do not heat.
- 3) Rinse original package with a small amount of water to remove all traces of powder. Add to solution in step 2.
- 4) For each litre being prepared, add 1.20g sodium bicarbonate (CAT N $^{\circ}$  : SPS-137-1KG) or 16.0 ml of 7.5% sodium bicarbonate solution (CAT N $^{\circ}$  : SLP-582-500). Mix until completely dissolved.
- 5) While stirring the solution, adjust the pH of the medium to 6.9 7.1 using 1 N HCl or 1 N NaOH. The pH of bicarbonate buffered solutions usually rises 0.1 0.2 units during filtration.
- 6) Add additional water to bring the solution to final volume.
- 7) Sterilize immediately by filtration using a membrane with a porosity of 0.22 microns.
- 8) Aseptically dispense medium into sterile container.