

## **Sperm BWW Stock Solution**

**Cat:** G2586 **Size:** 500mL

**Storage:** 2-8°C, valid for 1 year.

## Introduction

Normal semen is a kind of mixture, which is composed of the secretion of testis and epididymis and the sperm suspended in it and the secretion of prostate gland, seminal vesicle gland and bulbar gland of urethra during ejaculation. The resulting mixture is a viscous liquid. There are many methods of sperm analysis, which can detect by culture.

Sperm BWW Stock Solution is mainly composed of sodium chloride, potassium chloride, calcium chloride, magnesium sulfate, phenol red and so on. It do not contain glucose, sodium pyruvate, BSA and antibiotics. It is a common nutrient solution for capacitative treatment of broad-spectrum animal and human sperm cells.

## **Protocol**(for reference only)

- 1. Take a clean semen sample and place it at room temperature for 30-60min to make it fully liquefied.
- 2. Prepare sperm cells (for reference only, not necessary step):
  - 1) Upper stroke method: take a sterile 15ml conical bottom centrifuge tube and add 1ml liquefied semen, then gently add 1.2ml earle above the semen to form a liquid layer, tilt the centrifuge tube in 45°to increase the contact area of semen and culture solution. Incubate it at 37°C for 1 hour. Gently erect the test tube and suck the top 1ml of culture solution, which contains highly active sperm. Add 8ml of supplemental Earle culture solution to dilute, centrifugate in 300-500g for 5 min, and discard the supernatant. Add 0.5ml Earle culture solution and resuspend the cells for the evaluation of sperm density or function.
  - 2) Discontinuous density gradient method: take a sterile conical bottom centrifuge tube and add Percoll, then gently add 3ml 40% Percoll onto the liquid surface, operate carefully to avoid disrupting the interface between two liquids. Gently add 1-2ml semen to the gradient solution and discard the supernatant. Resuspend the sperm mass at the bottom of the tube in Earle culture solution, centrifuge and discard the supernatant. Add 1ml Earle culture solution and resuspend.
- 3. Incubate the centrifuge tube with sperm cells in a cell incubator containing 5% CO<sub>2</sub> and 95% air at 37 °C. If there is no such incubator, can seal and cover the centrifuge tube, then incubate it in an ordinary incubator at 37 °C. In the process of incubation, most of the motile sperm dissociate from seminal plasma to the culture medium covered above.
- 4. Centrifuge the sperm suspension to make the sperm cell density close to  $10 \times 10^6$ /ml. Resuspend the sperm cell in BWW Culture Medium and incubate in a cell incubator containing 5% CO<sub>2</sub> and 95% air at 37 °C. If there is no such incubator, can seal and cover the centrifuge tube, then incubate it in an ordinary incubator at 37 °C. During incubation, tilt the tube in 20°.

## Note

- 1. Pay attention to aseptic operation and try to avoid pollution.
- 2. If there is no Earle culture solution or supplementary Earle culture solution, can replace with Sperm BWW Culture Medium.
- 3. For your safety and health, please wear experimental clothes and disposable gloves.