

## **Extraction of total RNA from sperm of New Zealand white rabbits for detection of phospholipase C zeta (PLC $\zeta$ )**

### **ABSTRACT**

Fertilization is defined as the fusion of a spermatozoon to an ovum. Upon fusion, a sperm specific factor Phospholipase C  $\zeta$  was inserted triggering long lasting  $Ca^{2+}$  oscillations which drive egg activation and early embryonic development phase. Previous studies indicate that PLC  $\zeta$  homologues have been identified in humans, mice, pigs, monkeys and chickens. Although a preliminary study exhibits that PLC  $\zeta$  is present in rabbit testis, the sequence has not been published and the study on sperm has not been verified. Therefore, this study was conducted to isolate and detect PLC  $\zeta$  in rabbit sperm. Rabbit semen was collected using the artificial vagina. The semen was purified by using EquiPure Bottom Layer to remove bacteria and unwanted components in the fresh ejaculate. Purified semen was centrifuged at 4000 rpm for 10 minutes to separate sperm and seminal fluid. The sperm pellet on the bottom of the tube was subjected to RNA extraction by using Easy Blue Total RNA Extraction Kit. The purity and concentration of total RNA was quantified. Amplification reaction was performed by using One-Step RT-PCR system. The size of target gene was determined by conducting gel electrophoresis and eventually the band detected on the gel was viewed under Alpha Imager<sup>TM</sup> 2200. The visible band strongly demonstrated that PLC  $\zeta$  is also present in rabbit sperm which play an important role to trigger calcium oscillations during fertilization.

**Keyword:** PLC zeta; Total RNA extraction; Rabbits; Sperm