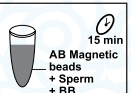
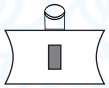
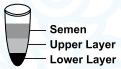
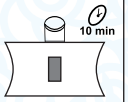
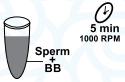
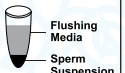
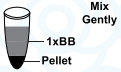
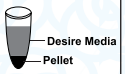
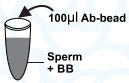
FLOW CHART

A) Reagent Preparation: • To prepare 1X, take 0.2ml from 10X stock and add 1.8ml sterile endotoxin free water.		8. Incubate the tube at room temperature (22-25°C) for 15 minutes.	
B) Sample Preparation: 1. Collect the sample in sterile collection jar. Allow to liquefy. Check count and motility.		9. After incubation, Place the tube in CANsep-Magnet Assembly.	
2. Wash the sample with double density gradient method.		10. Leave the tube in CANsep-Magnet Assembly at room temperature (22-25°C) for 10 minutes.	
3. Determine sperm concentration.		11. Aspirate the whole sperm suspension from the tube carefully **without removing the tube from magnet.**	
4. Take up to 50 X 10^6 sperm in sterile tube. Add 0.6 ml 1 X Binding Buffer Solution. Centrifuge the tube at 1000 RPM for 5 minutes.		12. Transfer the suspension in another sterile tube and add 2-3ml Flushing/ IVF media.	
5. Discard supernatant and to the pellet add 0.6ml 1 X Binding Buffer. Mix the content gently.		13. Centrifuge the tube at 1000 RPM for 10 minutes.	
6. Transfer the suspension in 6ml round bottom tube.		14. Discard the supernatant and finally resuspend the pellet in culture media for further ART procedure.	
7. Add 100 µl CANsep Nanobeads to the sperm suspension.			

CANDORE

REF : CA-003

CANsep

(Sperm Sorting Using Magnetic Separator)

CANDORE *Bioscience*

1st floor, Shashwat Complex, Naranpura,
Ahmedabad, Gujarat, India 380013.

Web: www.candore.in

E-mail: info@candore.in

Call: +91-982 405 7910, +91-79-2768 3121

Use by LOT Lot number REF Product reference Manufacturer Consult instruction of use

Introduction:

This is a non-invasive method to select non-apoptotic sperm using magnetic separation technology. Programmed cell death (Apoptosis) involves a series of morphological and biochemical changes that eventually result in cellular death. The extrusion of Phosphatidylserine (PS) which is normally present in the inner leaflet of the plasma membrane, to the outer surface has been identified as an early marker for apoptosis.

In this technique paramagnetic nanobeads conjugated with Annexin V, a 35-36 kDa phospholipid binding protein, which selectively binds to the external Phosphotidyl Serine (PS) present on the outer membrane of apoptotic sperm.

Reagents provided:

- CANsep Nanobeads
- 10 X Binding buffer solution

Storage and Stability:

- The kit must be stored at 4-8 ˚C temperature. Do not freeze.
- The reagent bottles should be tightly capped immediately after use.
- The kit reagents beyond the labeled expiration date should not be used for sample processing.

Precautions:

- All the samples and reagents must be treated as potentially hazardous.
- The solution and reagents must be discarded in a proper biohazard container at the end of the process.
- The performer must wear disposable gloves, eye protector and laboratory gowns while performing the process.

Procedure:

NOTE: ENSURE THAT ALL THE STEPS AND REAGENT PREPARATION ARE DONE UNDER STERILE CONDITION IN LAMINAR AIR FLOW.

A. Reagent Preparation

- Binding Buffer (1X): Take 0.2 ml of 10X stock solution and mix with 1.8ml of sterile endotoxin free water. Keep this solution at room temperature for 20 minutes before starting the procedure. Use this 1X working solution during the entire procedure.

B. Annexin V magnetic bead labeling protocol

Sample Preparation:

1. Collect the sample in the sterile semen collection jar. Allow the sample to get liquefy and check for count and motility.
2. Wash the sample preferably with Double Density Gradient method.
3. Determine the cell number after wash.
4. Take up to 50 x 10^6 sperms and add 0.6ml of 1x binding buffer in the sterile tube.
5. Centrifuge the cell suspension at 1000 RPM for 5 minutes. Aspirate the supernatant completely and discard the suppernatant.
6. To the pellet add 0.6ml of 1x working binding buffer solution and resuspend the pellet gently.
7. Transfer the suspension in sterile 6ml round bottom tube (Falcon Cat. No. 2003).
8. Then add 100µl of CANsep Nanobeads in the sperm- 1x binding buffer suspension and mix gently. Incubate the tube at room temperature (22-25˚C) for 15 minutes.
9. After completion of incubation, place the tube in CANsep Magnet Assembly and wait for 10 minutes.
10. Aspirate the cell suspension from the tube placed in magnet (DO NOT REMOVE THE TUBE FROM MAGNET BEFORE ASPIRATION) without interruption and touching the side walls of the tube and transfer in another sterile tube.
11. Add 2-3ml of flushing/ IVF media to the aspirated cell suspension and centrifuge the tube at 1000 RPM for 10 minutes.
12. Finally resuspend the sperm pellet in desire culture media and use for the appropriate ART procedure.

Recommendation during procedure:

- Perform all the steps under sterile condition as the final sperm suspension is used for further ART procedure.
- When sperm concentration is higher than suggested in procedure, increase the volume of all reagents proportionately. However, if the concentration of sperm is less than given in procedure use the volume given in the procedure.
- Sperm-magnetic bead labeling time and incubation time during magnet assembly is very crucial, so it is advisable not to exceed or decrease the specified time.
- Any interruption during the aspiration of sperm suspension during the tube placed in magnet assembly may lead to the amalgamation with the undesired sperm population. Aspirate the solution gently without any jerks.