For US Distribution Only

PICSI® Sperm Selection Device
Instructions for Use

Manufacturer: Biocoat, Inc., 211 Witmer Rd., Horsham, PA 19044 USA
Phone: 215-734-0888
Distributor: ORIGIO Inc., 2400 Hunters Way, Charlottesville, VA 22911
Phone: 434-979-4000
www.hba-picsi.com

Intended Use: In the treatment of infertile couples by ICSI, PICSI® is indicated for the selection of mature sperm for injection.

Principle of the Device: Hyaluronan is a main component of the *cumulus oophorus* layer that surrounds the oocyte. The head of a mature sperm carries a hyaluronan-specific receptor that enables mature sperm to bind to hyaluronan (1). In contrast, **immature sperm do not bind**. Mature sperm exhibit a high DNA chain integrity (2), a normal frequency of chromosomal aneuploidies and provide a paternal contribution to the zygote comparable to that of sperm selected by the zona pellucida during natural fertilization (3).

In the usual practice of ICSI, sperm are visually selected for injection on the basis of their morphology and motility. However, this approach does not reflect the genomic integrity of the sperm and its ability to provide the best paternal contribution to the zygote. The PICSI® Sperm Selection Device provides a means to select mature sperm based on their ability to bind to hyaluronan hydrogel. The PICSI® Sperm Selection Device mimics the natural binding of mature sperm to the *cumulus oophorous*, an important selective step in natural fertilization.

Description: The PICSI® Sperm Selection Device is a polystyrene culture dish with three microdots of hyaluronan attached to the interior bottom. Three locating lines embossed on the bottom exterior of the dish facilitate the location of the microdots. The microdot is found in an area approximately 2 mm wide and 3 mm long projecting from the end of the locating line. The device is sterile, Sterility Assurance Level 10^{-6}, pyrogen-free (<1 EU/device), and has been Mouse Embryo Assay tested (1-Cell MEA >75% blastocysts 96 h).

Preparation for use: Hydrate the hyaluronan microdots by placing single 10-µL droplets of Human Tubal Fluid (HTF) containing at least 5 mg/mL serum protein, or other suitable sperm diluent, at the end of each locating line covering the area where the microdot is situated (Figure 1). Alternatively, the sperm suspension can be added directly to the dry microdot. Drops of polyvinylpyrrolidone (PVP) or other fluids useful for manipulating sperm may also be placed elsewhere on the dish at this time. Carefully flood the dish with tissue culture oil to prepare it for use.

Hydrating the microdot before applying the sperm gives the hyaluronan time to swell. Swelling and sperm binding begin normally in 5 minutes or less. However some microdots may require 30 minutes or more to reach full binding capability. Therefore, whenever marginal sperm binding is observed, pre-hydrate for 30 minutes or more, or allow sperm to incubate on the dot for 30 minutes or more before selecting sperm.

Using the PICSI® Sperm Selection Device: Add the sperm to the pre-hydrated microdot in a volume equal to or greater than that used to pre-hydrate the dot (approximately 10 µL). Touch the tip of the micropipette containing the sperm to the edge of the hydrating drop at the bottom of the dish under the oil and expel the sperm. By delivering the sperm in a volume equal to the hydrating fluid, immediate mixing and delivery of sperm to the vicinity of the microdot is assured. If the sperm are delivered in a smaller volume at the edge of the drop, greater than 30 minutes may be required for them to swim through the hydrating fluid to the microdot. Once bound, hyaluronan-bound sperm are easily identified: they exhibit no progressive migration despite vigorous tail beating.

Factors governing sperm binding: To rapidly populate the microdot with bound sperm, place approximately 100,000 hyaluronan-binding sperm per mL (approximately 1,000-2,000 total sperm in 10-20 µL volume) over the microdot, see Figure 2. As time passes, the number of bound sperm will increase as more swimming sperm make contact with the hyaluronan microdot.

Sperm Location Selection: The wall of the hyaluronan microdot is a physical barrier to which many sperm will bind since this is usually the first point of contact. It is sometimes difficult to distinguish whether the sperm are bound or they are simply swimming against the edge of the microdot. You may be sure of selecting bound sperm by selecting them from the **interior** of the microdot.

Obtaining a good density of bound sperm: If the density of bound sperm is too high or too low for good sperm selection, dilute or concentrate the prepared sperm sample and use the adjusted sperm sample to seed the next microdot. Three microdots are provided on each PICSI® Sperm Selection Device to give a sufficient opportunity.

Sperm collection: To collect a bound sperm, position the tip of the ICSI micropipette next to the sperm and gently suck fluid into the pipette, drawing in the sperm. Continue collecting until 20-50 sperm are captured. Expel the captured sperm into a PVP drop to process them for ICSI (inactivating the tail, re-evaluating motility and morphology.) From the PVP droplet, select and load single, processed sperm for injection into the oocytes according to your standard injection protocol.

Temperature: Sperm bind best to hyaluronan hydrogel at temperatures below 30°C. At temperatures above 30°C, sperm swimming vigor increases and the swimming force may overcome the binding force. The result is that about one-third of sperm bound at room temperature will show some progressive migration at 37°C and may be deemed not bound, immature. PICSI® Sperm Selection Device dishes placed on a 37°C heated stage will come to about 33°C and then remain at that temperature. At 33°C or even at 37°C, many bound sperm will remain available for selection.

Technique considerations: Microdot shape: The PICSI® Sperm Selection Device hyaluronan microdot is crater-shaped. The edge of the microdot is a raised wall of hydrogel surrounding a low, flat interior layer. The wall is flexible and may be irregular in shape due to uneven hydration of the hydrogel.

The hydrogel wall can be pierced and torn by an ICSI micropipette driven directly into it. It is best to position the elevated micropipette tip over the microdot interior and lower it to the microdot surface for recovery of sperm.

Microdot caves: During manufacture, uneven hydration may cause segments of the microdot wall to create small "caves" that open toward the inside edge of the wall. Sperm that swim into a cave are trapped, not bound. Trapped sperm usually all face away from the center of the microdot and show vigorously beating tails, often in clusters. The heads of trapped sperm can move laterally and sometimes back and forth within the walls of the cave. Trapped sperm should not be selected since their binding status is unclear.

Microdot stability: If a part of the wall separates from the polystyrene, the same forces that create caves can cause the microdot wall to progressively detach from the dish and coil up like a spring. When this occurs, some or all of the wall will separate from the microdot. However, the microdot interior hyaluronan layer will remain intact. The interior hyaluronan layer is stable for hours, it collects and houses bound sperm that may be used for ICSI. Sperm bound to the curled up wall remnant should not be used for sperm selection and isolation.

Troubleshooting: If sperm do not bind to the microdot:

1. Determine that the sperm sample contains mature, hyaluronan-binding sperm by assaying with an HBA® Hyaluronan Binding Assay. If the HBA score is near zero (<5%), no sperm binding is expected (In some laboratories, as much as 10% of the donor sperm population shows no sperm binding. This is a significant factor contributing to infertility).
2. How long have the sperm been incubated on the microdot? Binding is directly related to the time allowed for binding. Sperm bind when they make contact with the hyaluronan microdot, swimming randomly. The number of sperm bound to the dot will grow with time as more sperm encounter the hyaluronan microdot. Check the microdot periodically over two hours to see if sufficient bound sperm have accumulated for an ICSI procedure.
3. The density of hyaluronan-binding sperm is critical. Rapid population of the microdot requires a density of at least 100,000 hyaluronan-binding sperm per mL. If the HBA score is low, the total sperm density must be increased to deliver an effective number of hyaluronan-binding sperm. For example: if the HBA score is 10%, 1,000,000 total sperm per mL will be required to deliver 100,000 hyaluronan-binding sperm per mL.

Warning: U.S. Federal law restricts this device to the sale by, or on the order of, a physician. A Certificate of Analysis, if not already provided with the shipment, is available on request from the distributor. **This device is covered under US patent 5,897,988 and the corresponding foreign patent applications.**

Questions or Comments: Please contact the Distributor at the telephone or via the internet address given above.

References:

1. Huszar, G., *et al*. 2003. Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. *Fertil. Steril.* **79**(3):1616-24.
2. Yagci, A., W. Mark, J. Stronk and G. Huszar. 2010. Spermatozoa bound to solid state hyaluronic acid show chromatin structure with high DNA chain integrity: an acridine orange fluorescence study. J. Androl. **31(6):**566-72.
3. Jakab, A. *et al*. 2005. Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies. *Fertil. Steril.* **84**(6):1665-73.

Figure 1, the PICSI® dish.

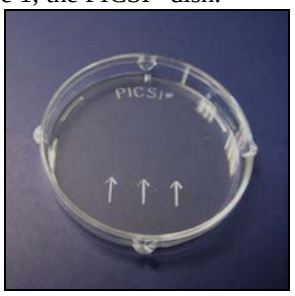
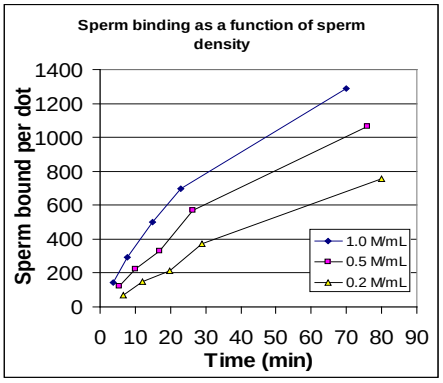


Figure 2, Sperm binding versus time and sperm density. (M = 1,000,000)



Labeling Part #:283-200
Rev.: B