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Morbid and DNA fragmented Sperm depletion Nanoparticles

cell sorting reagent

COMPOSITION

Magnetite Fe Coating: Dextran 18K molecular Weight Dextran/ Fe

Conformation of coating: Microscopic evaluation and confirmation

Glycoroteins in the Coating: PNA / LCA / Annexin V

Sterility: All solutions prepared are sterile filtered using 0.2 micron filters

pH: USP current edition pH range 7.2-7.4 actual pH 7.4

Suspension Media: 10 mM Potassium phosphate buffer

Osmolality: 280 mOsm/l

MECHANISM OF ACTION FOR EACH LIGAND USED

Peanut Agluttinin (binds to Gal-β(1-3)-GalNAc motif which helps identify acrosome and sperm acrosome integrity)

Blumer, Camile Garcia; Restelli, Adriana Ester; Giudice, Paula Toni Del; Soler, Thiesa Butterby; Fraietta, Renato; Nichi, Marcilio; Bertolla, Ricardo Pimenta; Cedenho, Agnaldo Pereira. "Effect of varicocele on sperm function and semen oxidative stress". BJU International. 109 (2): 259–265. doi:10.1111/j.1464-410X.2011.10240.x.

LCA (binds to Fucosylated core region of bi- and triantennary complex type N-Glycans motif has an important role in sperm motility)

Xin, A.-J., Cheng, L., Diao, H., Wang, P., Gu, Y.-H., Wu, B., … Tao, S.-C. (2014). Comprehensive profiling of accessible surface glycans of mammalian sperm using a lectin microarray. Clinical Proteomics, 11(1), 10. http://doi.org/10.1186/1559-0275-11-10

Annexin V (Annexin A5 is considered a non-quantitative probe used to detect cells that have expressed phosphatidylserine motif on their cell surface where PS is found on cell surface in apoptosis as well as other forms of cell death)

Meers P and Mealy T (1994). "Phospholipid determinants for annexin V binding sites and the role of tryptophan". Biochemistry. 33 (19): 5829–37. doi:10.1021/bi00185a022.

PREPARATION

-The reagent requires washing from sodium azide preservative before application to washed

spermatozoa.

REAGENTS

-Solution of Fe salts B process with dextran 18K molecular weight coating with concentration 110mg

dextran per 780 mg Fe.

STERILITY

-All solutions prepared are sterile filtered using 0.2 micro filters

PRECAUTIONS

-Do not ingest.

-For In vitro use only.

STORAGE AND STABILITY

-Product is stable in room temperature and light.

-Store at 2-4 °C , do not freeze under any circumstance.

SHELF LIFE

Product shelf life is 6 months from production date, Particles can still be used after 6 months for 3 more months as long as the color of the solution haven't changed to reddish brown (oxidized). However, the shed amount of particles will be higher which would reduce the overall efficiency of the product.

SAMPLES

For application on washed spermatozoa only.

PROCEDURE

REMOVAL OF Sodium Azide (washing steps)

-Hold tube with magnetic particles against the magnet (preferably with a rubber band or in a rack) until the particles gather against the wall of the tube. Normally within 4 minutes.

-Decant or aspirate out the supernatant while the particles are still in the magnetic field and held against the wall of the tube.

-Remove magnet and resuspend the particles with the desired washing buffer. Gently invert the suspension of particles and liquid until particles are dispersed

-Repeat step one again

-Resuspend the particles as in step 2 and 3.Particles are ready for use

Procedure for ICSI

1-Prepare sperm by either washing with either Hepes washing buffer, extender HTF with or without BSA or Density Gradient Centrifugation.

2-For one dose add 225 ul of particles to the 5 million sperm/ml (1 ml containing 5M + the 225 ul particle suspension).

3-Gently mix the particles and sperm for 30 minutes at room temperature.

4-Place the particle sperm solution against the magnet for 10 minutes. Decant the supernatant while the particles are still against the wall of the tube and magnet. (The ready to use sperm are in the supernatant)

5- proceed to ICSI with the supernatant.

Procedure for IVF

1-Prepare sperm by either washing with either Hepes washing buffer, extender HTF with or without BSA or Density Gradient Centrifugation.

2-For one dose add 225 ul of particles to the 5 million sperm/ml (1 ml containing 5M + the 225 ul particle suspension).

3-Gently mix the particles and sperm for 30 minutes at room temperature.

4-Place the particle sperm solution against the magnet for 10 minutes.

5-Decant the supernatant while the particles are still against the wall of the tube and magnet. (The ready to use sperm are in the supernatant).

6-proceed to IVF with the supernatant.

INTERFERENCES

-Some antibiotic or antifungal agents could hinder the product efficiency in case of storage.

-Do not use with Bicarbonate buffers as well as buffers requiring PH stabilization prior to use.

-Recommendation: use with PBS or HEPES.

NOTES

-Upon receipt of Nano particles refrigerate at 4 0 C. Do not freeze

-Before using the NP make sure the particles are re suspended by gently mixing by hand

-Removal of Sodium Azide from the particle suspension if present

BIBLIOGRAPHY

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and health assessments of produced offspring

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3. Assessment of magnetic nanoparticle exposure on sperm motility and fertility potential

https://www.researchgate.net/project/nano-treated-pig-sperm-with-improved-fertility

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https://www.ncbi.nlm.nih.gov/pubmed/15866582

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8. Utility of Magnetic Cell Separation as a Molecular Sperm Preparation Technique.
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