

Flow Cytometric Analysis of Biomarkers for Detecting Human Sperm Functional Defects

 Xi Ling¹, Peng Zou¹, Lin Ao¹, Niya Zhou¹, Xiaogang Wang², Lei Sun¹, Huan Yang¹, Jinyi Liu¹, Jia Cao¹, Qing Chen¹
¹Institute of Toxicology, College of Preventive Medicine, Army Medical University (Third Military Medical University) ²Department of Chemical Defense Medicine, College of Military Preventive Medicine, Army Medical University (Third Military Medical University)

Corresponding Author

 Qing Chen
 chenqingforward@gmail.com

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Materials

Name	Company	Catalog Number	Comments
0.1 M citric acid buffer	Sigma-Aldrich Chemical Co., USA	251275-5G	Add 21.01 g/L citric acid monohydrate (FW = 210.14; 0.10 M) to 1.0 L H ₂ O. Store up to several months at 4 °C.
0.2 M Na ₂ PO ₄ buffer	Sigma-Aldrich Chemical Co., USA	V900061-500G	Add 28.4 g sodium phosphate dibasic (FW = 141.96; 0.2 M) to 1.0 L H ₂ O. Store up to several months at 4 °C.
10 mM CCCP stock solution	Sigma-Aldrich Chemical Co., USA	C2759	Add 20.46 mg CCCP to 10.0 mL DMSO, making up to the final of CCCP in a concentration of 10mM, aliquot and store at -80 °C.
10 μM CCCP working solution			Add 10 mL of PBS to a 15 mL polypropylene centrifuge tube and add 10 μL CCCP stock solution, making up to the final of CCCP in a concentration of 10 μM.
1 mM JC-1 stock solution	Sigma-Aldrich Chemical Co., USA	T4069	JC-1 is purchased lyophilized. Add a small quantity of DMSO to the vial and vortex for several minutes until all the dye has dissolved. Transfer the solution to a light-tight tube and rinse the vial with appropriate volume of DMSO, making up to the final of JC-1 in a concentration of 1 mg/mL aliquot and store at -20 °C.
37 °C incubator	Thermo Scientific, USA		
5 μM JC-1 working solution			Add 10 mL of PBS to a 15 mL polypropylene centrifuge tube and add 50 μL from a thawed JC-1 stock aliquot. Stir gently to assure a homogenous dilution. This solution must be stored in the dark and used promptly.
Accuri C6 Flow cytometer	BD Pharmingen, San Diego, CA, United States		

Acid solution, pH 1.2			Combine 20.0 mL of 2.0 N HCl (0.08 N), 4.39 g of NaCl (0.15 M), and 0.5 mL of Triton X-100 (0.1%) in H ₂ O for a final volume of 500 mL. Adjust pH to 1.2 with 5 mol/L HCl.
Acridine Orange (AO) stock solution, 1.0 mg/mL	Polysciences, Inc, Warrington, Pa	65-61-2	Dissolve chromatographically purified AO in dd-H ₂ O at 1.0 mg/mL.
AO staining solution (working solution)			Add 600 µL of AO stock solution to each 100 mL of staining buffer.
Biological safety hood	Airtech, USA		
Computer-aided sperm analysis system (CASA)	Microptic, Barcelona, Spain		Sperm Class Analyzer 5.3.00
Sperm Counting Chamber	Goldcyto, Spain		
Equipments			
FITC Annexin V Apoptosis Detection Kit I	BD Biosciences, San Jose, CA	556547	Included: (1) FITC Annexin V is bottled at 100 ng/µL; (2) Propidium iodide (PI):The PI Staining Solution is composed of 50 µg PI/mL in PBS (pH 7.4) and is 0.2 µM sterile filtered; (3) 10x Binding Buffer: 0.1 M Hepes (pH 7.4), 1.4 M NaCl, 25 mM CaCl ₂ . For a 1x working solution, dilute 1 part of the 10x Annexin V Binding Buffer to 9 parts of distilled water. Store at 4 °C and protected from prolonged exposure to light. Do not freeze.
FlowJo 10	Tree Star, Inc., San Carlos, CA, USA		
Horizontal centrifuge	Thermo Scientific, USA		
liquid nitrogen tank	Thermolyne, USA		
Materials			
PBS	Beyotime, Shanghai, China	C0221A	Ready-to-use PBS buffers is purchased and stored at room temperature
Staining buffer, pH 6.0			Combine 370 mL of 0.10 M citric acid buffer, 630 mL of 0.20 M Na ₂ PO ₄ buffer, 372 mg of EDTA (disodium, FW = 372.24; 1 mM), and 8.77 g of NaCl (0.15 M). Mix overnight on a stir plate to insure that the EDTA is entirely in solution. pH to 6.0 with saturated NaOH solution.
The reference sample for SCSA analysis			The reference sample was diluted with cold (4 °C) TNE buffer to a working concentration of 1–2 × 10 ⁶ cells/mL, and used to set the green at 475/1,024 flow cytometry channels and set the red at 125/1,024 flow cytometry channels.
TNE buffer, 1x, pH 7.4 (working solution)			Combine 60 mL of 10x TNE and 540 mL of H ₂ O. Check pH (7.4).
TNE buffer, 10x, pH 7.4	Perfemiker, Shanghai, China	PM11733	Ready-to-use buffers is purchased and stored at 4 °C.