

Uninterrupted Culture Media Complete Recommended Protocol

Continuous Single Culture-NX Complete (CSCM-NXC) and Continuous Single Culture Complete (CSCM-C)

Microdrop

CSCM-NXC and CSCM-C are optimized to be used in a uninterrupted culture system without medium renewal or dish change on Day 3.

MEDIUM PREPARATION

CSCM-NXC and CSCM-C are complete, ready-to-use media containing Human Serum Albumin (HSA) as the protein component. It is not necessary to add any other protein before use.¹

DISH PREPARATION


A sufficient volume of CSCM-NXC or CSCM-C is required so that oocyte collection, insemination, and embryo culture dishes can be prepared.

Oocyte Collection and Insemination Dishes (60 x 15 mm):

On the day before oocyte retrieval (Day -1) prepare dishes with eight 100 μ L volume drops of pre-supplemented uninterrupted culture medium along the perimeter of the dish with an 11 mL volume oil overlay.

Embryo Culture Dishes (60 x 15 mm): On the day of oocyte collection (Day 0), prepare microdrop dishes by aseptically dispensing at least six 50–100 μ L drops of pre-supplemented uninterrupted culture medium into the dish. Four microdrops should be placed at the 3, 6, 9 and 12 o'clock position and the two remaining drops should be placed in the center of the dish to be used as wash drops. 11 mL's of Oil for Embryo Culture should be used to completely cover the uninterrupted culture medium microdrops. To avoid evaporation and changes in osmolality do not prepare multiple dishes at the same time. Immediately place the dish in a CO₂ incubator.

Time-lapse Surveillance System: CSCM-NXC and CSCM-C work effectively in all time-lapse surveillance systems and should be used accordingly to the individual system's protocol.


 To ensure proper temperature and pH of medium and oil, all dishes should be pre-equilibrated overnight to 37°C in a 5–6% CO₂ incubator on the day before oocyte collection (Day -1) and on the day of oocyte collection (Day 0). Minimum incubation time should be no less than 6 hours.

CO₂ AND PH

We recommend using reduced oxygen when possible; however, CSCM-C can be used in ambient oxygen incubators. Following CO₂ equilibration, the pH of the uninterrupted culture medium should be within the acceptable pH range of 7.25–7.40 with a targeted pH of ~7.26–7.35.

Tri-gas Incubator: Ideally, we recommend 6% CO₂, 6% O₂, and 88% N₂ for optimal fertilization and embryo growth at or near sea level.

CO₂ Incubator: Alternatively, we recommend a 5–6% CO₂ for sufficient fertilization and embryo growth at or near sea level.

 pH levels are affected by the altitude above sea level (elevation). As the elevation increases the gas pressure over the culture medium decreases requiring a higher CO₂ pressure to achieve the optimal pH for the uninterrupted culture medium (7.26–7.35). To adjust pH, increase CO₂ levels to lower pH and decrease CO₂ levels to raise pH to fall within the specified range. pH should always be verified with pH measurements using a pH meter.

OOCYTE RECOVERY

Immediately upon oocyte collection and identification, it is recommended that oocytes be placed into the pre-equilibrated oocyte collection dish (prepared on Day -1). Following the trimming of the cumulus coronal complex from each oocyte, place 1-3 oocytes into a fresh drop of the oocyte collection dish and return to the controlled gas incubator at 37°C for the desired recovery period. Oocytes should be incubated for a minimum of 1 hour prior to insemination, or denuding if ICSI is to be performed. Oocytes should remain in the collection dish for up to 4 hours prior to conventional IVF or insemination by Intracytoplasmic Sperm Injection (ICSI).

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INSEMINATION OF OOCYTES FOR CONVENTIONAL IVF


Upon oocyte recovery, trimming of cumulus complex, and incubation for up to 4 hours (minimum 1 hour), remove the oocytes from the collection dish and transfer the oocytes to the pre-supplemented uninterrupted culture medium droplets in the pre-equilibrated insemination dish (prepared on Day -1). It is recommended to aseptically dispense 75,000–175,000/mL motile sperm per micro droplet containing 1–3 oocytes. Return the insemination dish to the incubator and check for normal fertilization 16–20 hours post insemination.

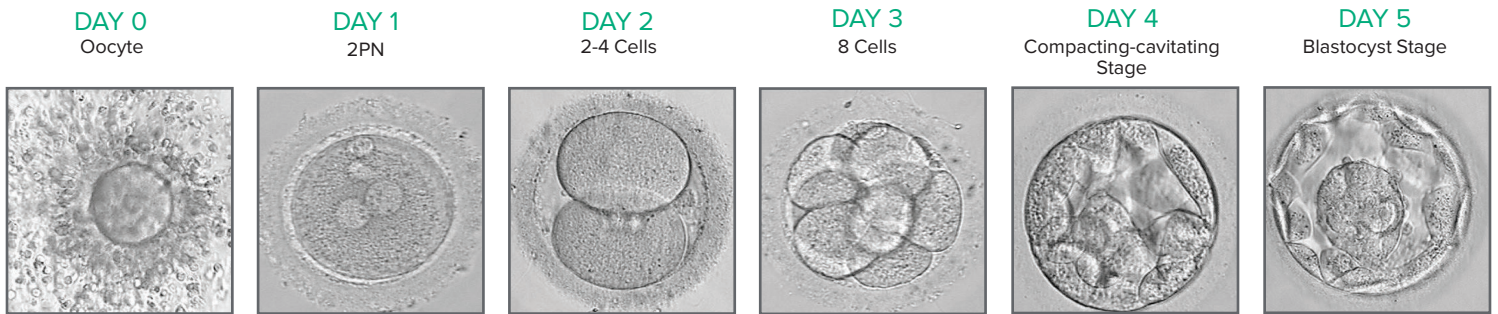
ICSI

Following >1 hour post oocyte denuding, remove denuded oocytes from incubator and inseminate with sperm as per standard ICSI protocol. Immediately following insemination, place 1–3 inseminated oocytes into a fresh drop of the pre-supplemented uninterrupted culture medium in the pre-equilibrated insemination dish (prepared on Day -1), and return dish to the incubator and check for normal fertilization 16–20 hours post insemination.

EMBRYO CULTURE POST FERTILIZATION

Following fertilization assessments with the identification of the presence of normal fertilization (two pronuclei and two polar bodies), transfer 2–5 2PN zygotes into each of the four pre-equilibrated 50–100 μ L micro drops in the culture dish previously prepared on Day 0. We recommend embryos to be group cultured (maximum 5 embryos per micro drop) in a static, uninterrupted culture system without dish change or medium renewal on Day 3, until the desired stage of embryo development is achieved.

 5 is the maximum number of embryos that we recommend being cultured in each 50–100 μ L micro drop.



1. FUJIFILM Irvine Scientific has not validated these procedures and each laboratory should consult its own laboratory procedures and protocols which have been specifically developed and optimized for your individual medical program.

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FUJIFILM IRVINE SCIENTIFIC – CORPORATE
1830 E Warner Avenue, Santa Ana, CA 92705 USA
Phone: 1 (949) 261-7800
Toll Free: 1 (800) 437-5706
Fax: 1 (949) 261-6522
Support: tmrequest@irvinesci.com