FUJIFILM
Value from Innovation

IrvineScientific

MEGA Assay

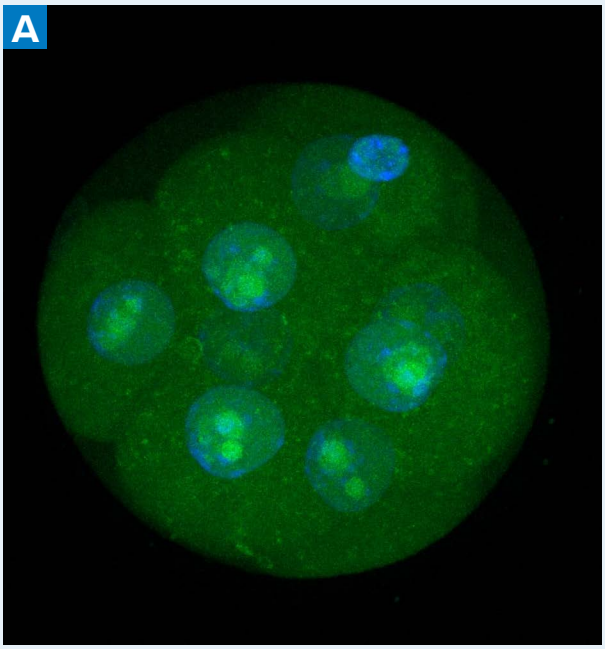
Modernizing quality control in IVF

MEGA assures product consistency through:

- Analysis of pre-implantation embryo development and health by gene expression in addition to morphology
- Increased sensitivity for detecting embryotoxicity in raw materials prior to manufacture
- Assessment of efficacy at early stages of development

The mouse embryo assay (MEA) has long been used as the standard method for screening raw materials, media, and labware used in IVF clinics. However, the sensitivity of the MEA to detect cytotoxicity in materials has been questioned for many years. Organizations such as ASRM and ESHRE, as well as numerous publications, have called for a more relevant and sensitive test than MEA[1,2,3]. Furthermore, being based solely on morphology at a single point in time, the MEA is inherently limited in its capability.

FUJIFILM Irvine Scientific has developed a genetic mouse embryo assay called MEGA. Using embryos from a genetically-engineered mouse strain, MEGA employs fluorescence-based detection of developmentally-regulated gene expression to assess embryos throughout the development stages including at the earliest stages of growth. The result is a bioassay that is more sensitive to embryotoxic materials and provides more functional information at an earlier stage of development.



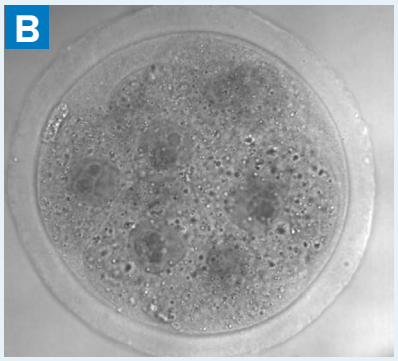


Figure 1. A healthy mouse embryo expressing Oct4-GFP at 48 hours **(A)**. The same embryo displayed healthy morphology at 48 hours **(B)** to 96 hours **(C)**. The blue fluorescence reflects DAPI staining of the nucleus of each cell.

Reduce concerns about toxicity with the power of genetic engineering

MEGA employs a functional molecular biomarker to improve the detection of toxic media or any other environmental stress that may negatively impact early development. The assay measures the level and location of fluorescence produced by green fluorescent protein (GFP), which is expressed under the control of the Oct4 promoter (also known as *Pou5f1*). Regulation of Oct4 expression is one of the most critical early indicators of normal embryonic development. Combined with traditional morphological evaluations, MEGA monitors both the early and late stages of development.

Unlike the traditional MEA, MEGA can harness the power of genetic engineering to further enhance and refine our capability of improving assisted reproductive technologies (ART)/IVF media and products.

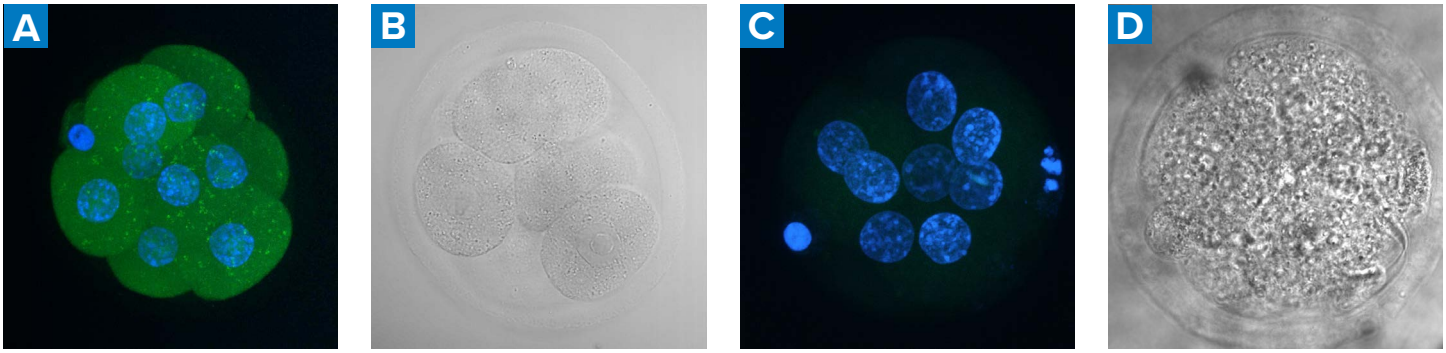


Figure 2. Normal expression of Oct4-GFP **(A)** during early embryogenesis at 48 hours reflects healthy development in optimal culture conditions, corroborated by morphology **(B)**. Compare this to low expression of Oct4-GFP seen in suboptimal conditions **(C)** even though morphology appears normal **(D)**. MEGA facilitates early detection of embryotoxicity and abnormal embryo development. The blue fluorescence reflects DAPI staining of the nucleus of each cell.

Why MEGA was developed

With the use of *in vitro* fertilization (IVF) and other assisted reproductive technologies (ART) expanding worldwide, safety is an increasingly important concern. Regulatory bodies, clinics, and patients have raised their expectations on quality control and raw material sourcing. MEGA was developed to address these expectations.

As a manufacturer of IVF media, one of our primary goals is to apply the most rigorous quality control processes available to evaluate raw materials. This process is particularly important for complex components such as oil and Human Serum Albumin. With MEGA, our ability to detect suboptimal components is significantly improved. Further enhancements to MEGA, afforded by new strains of genetically modified embryos, may enable additional improvements to the reagents and materials used in ART/IVF.

Improving quality control through raw material qualification with MEGA

FUJIFILM Irvine Scientific operates a robust raw material and supply chain qualification process. Stringent testing of raw materials using the MEGA assay prior to their use in finished products provides extra assurance of quality.

MEGA Embryo Preparation

Embryos expressing Oct4-GFP (one-cell stage) are harvested from transgenic mice

Fresh embryos with normal morphology and 2 pronuclei are pooled and randomly allocated for test and control conditions

Raw Material Preparation

High risk raw materials received from qualified vendors prepared for testing (HSA, alpha beta globulin and oil)

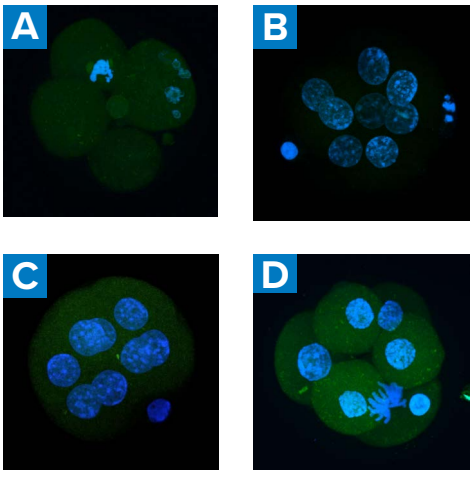
- Test HSA is prepared with control media
- Test α β-globulin is prepared with control HSA and media
- Test and control oil will be laid over control media

MEGA Assay run

Petri dishes with 20 µL droplets of culture media are prepared with a mineral oil overlay. Embryos are placed into test and control media (overlaid with test or control oil) for 96 hour incubation.

Results analyzed over 96 hours

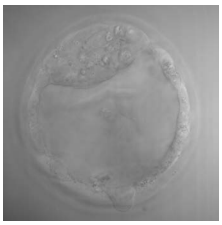
At 48 hours



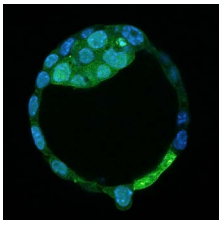
Early fluorescent intensity (EFI) is assessed and embryos are ranked as having sub-normal **(A, B)** or normal **(C, D)** expression levels.

Culture conditions resulting in lower proportions of normal-EFI embryos are considered abnormal or suboptimal.

At 96 hours



Embryos checked for normal development into blastocysts



Oct4-GFP expression is localized to the inner cell mass

Approved raw materials used to manufacture final products

Finished products undergo additional quality control testing

MEGA shows increased sensitivity to detect suboptimal quality and toxicity

EFI eliminated suboptimal quality protein in the screening of 5 different HSA lots

To demonstrate the added sensitivity of MEGA, Human Serum Albumin (HSA) was tested across five different lots (n=43 embryos per lot). While all lots showed no differences for 8-cell morphology at 48h with standard MEA, MEGA detected suboptimal performance of 2 lots (A and D) from their lower early fluorescence intensity (EFI). Subsequently, lot A was rejected based on morphology (<80% blastocysts) and lower EFI, while lot D was rejected based on lower EFI (morphology marginally passed)[4].

	At 48 hr		At 96 hr
Lot #	≥ 8-cell morphology (%)	High EFI (%)	96 hr E/H Blastocysts (%)
HSA-A	95.5	86.4	72.7
HSA-B	95.3	90.7	86
HSA-C	97.7	93	93
HSA-D	97.7	81.4	83.7
HSA-E	95.3	95.3	95.3

Table 1. Early Fluorescence Intensity at 48h was predictive of early/hatching blastocyst rates at 96h when MEGA was used to screen lots of HSA. Red highlights indicate lower proportion of normal EFI and corresponding lower or marginal E/H rates of suboptimal lots of protein that would be rejected by MEGA quality control testing.

MEGA is more sensitive at detecting toxicity in oil than standard MEA

An oil dose-response experiment was used to assess the sensitivity of MEGA to detect embryotoxicity and suboptimal culture conditions. Pou5f1-GFP embryos cultured under oil overlays containing 0 (control), 5 or 10% previously identified adulterated (PID) oil were evaluated for embryo expression (EFI) at 48 hours and development at 96 hours (E/H blastocysts).

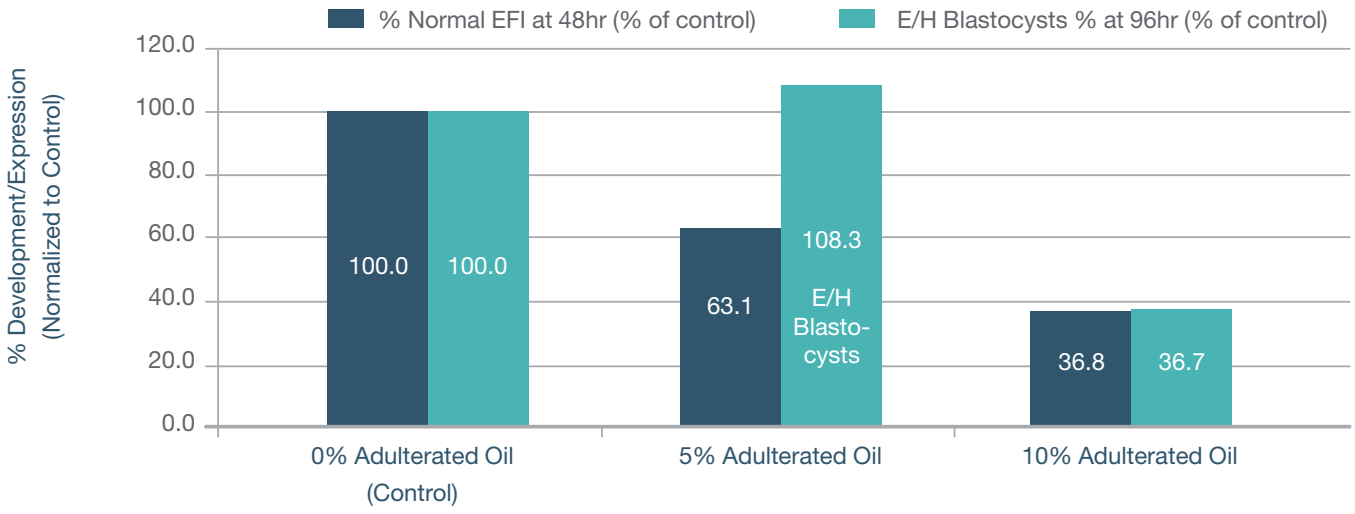


Figure 3. Morphology alone was less sensitive than EFI in detecting PID oil. Morphology was a good indicator in the 10% condition but did not detect toxicity in the lower 5% condition. EFI at 48hr did detect the toxic component in the 5% PID condition showing increased sensitivity compared to morphology. MEGA has repeatedly demonstrated similar efficacy during assay development[4].

1. Gianaroli et al. ASRM Pages. 2012 Dec;98:6. 2. Elder et al. In-Vitro Fertilization. 2010 Dec. 3. Mortimer et al. Quality and Risk Management in the IVF Laboratory. 2015 Mar.
4. Gilbert et al. Reprod Biol Endocrinol. 2016 Mar;14:13. doi: 10.1186/s12958-016-0149-x.

MEGA is part of our commitment to quality

Quality through scientific innovation is a core tenet at FUJIFILM Irvine Scientific. MEGA was developed to meet customer expectations for media of consistently high quality, and to create a new standard of embryotoxicity detection that would allow further improvements in ART/IVF.

Strict testing of raw materials is just one facet of the quality control systems at FUJIFILM Irvine Scientific. We were one of the first ART manufacturing companies in the USA to receive ISO 13485:2003 certification of our Quality System. Products are manufactured in accordance with the Current Guidelines for Manufacture of In Vitro Diagnostic Products and the Good Manufacturing Practices (cGMP) for Medical Devices.

Formulations use WFI Grade Water and chemicals that meet USP and ACS standards where available. All products are membrane-filtered and aseptically processed according to manufacturing procedures which have been validated to meet sterility requirements according to USP <71> Sterility Test requirements. Every batch of medium is thoroughly tested for functionality, endotoxin level pH, osmolality, and sterility. Results are provided in lot-specific Certificates of Analysis.

With the exception of a small subset of ancillary products, all key products for IVF are CE marked and FDA cleared. Quality and regulatory management systems ensure compliance with the broadest scope of regulatory requirements, including ISO 13485, FDA's QSR, and the European Medical Devices Direction (93/42/EEC).

For more information about MEGA or our products that benefit from these rigorous quality control processes, contact us at **getinfo@irvinesci.com** or visit our website at **www.irvinesci.com/contact-us**

Raw materials tested by MEGA are used in:

- Human Serum Albumin
- Serum Substitute Supplement
- Oil for Embryo Culture
- Heavy Oil for Embryo Culture
- Media supplemented with protein

Ordering Information

Protein Supplements

Item	Catalog #	Size	Additional Information	Shelf Life*	Storage
Human Serum Albumin (HSA)	9988	12 × 5 mL 100 mL	Saline solution containing total protein 10% w/v, 100% HSA.	3 years	2–8°C
Serum Substitute Supplement (SSS)	99193	12 × 12 mL 100 mL	Consists of Human Serum Albumin from therapeutic-grade source material and human serum globulins in saline solution.	2 years	2–8°C

Oil for Embryo Culture

Item	Catalog #	Size	Additional Information	Shelf Life*	Storage
Heavy Oil for Embryo Culture	90189	100 mL 500 mL	Ready-to-use sterile heavy mineral oil overlay for small media volumes	2 years 8 weeks after opening	2–8°C
Oil for Embryo Culture	9305	100 mL 500 mL	Ready-to-use, sterile, light mineral oil.	2 years 8 weeks after opening	15–30°C

Media Containing Proteins Qualified by MEGA

Item	Catalog #	Size	Additional Information	Shelf Life*	Storage
Continuous Single Culture Complete (CSCM-C)	90165	2 × 20 mL	Ready-to-use, pre-supplemented with Human Serum Albumin (5% v/v HSA), for a final total protein concentration of 5 mg/mL.	120 days 8 weeks after opening	2–8°C
Continuous Single Culture- NXC (CSCM-NXC)	90168	2 x 20 mL	Ready-to-use, pre-supplemented with Human Serum Albumin (5% v/v HSA), for a final total protein concentration of 5 mg/mL.	120 days 8 weeks after opening	2–8°C
Multipurpose Handling Medium– Complete (MHM-C)	90166	100 mL 500 mL 12 × 12 mL**	Ready-to-use. Contains key amino acids, 0.5 % HSA, gentamicin 10 mg/L, and is dual buffered with HEPES and MOPS.	1 year 5 weeks after opening 7 days after opening**	2–8°C
Vit Kit-Freeze	90133-SO	ES, 2 × 1 mL VS, 2 × 1 mL	For use with oocytes (MII), pronuclear (PN) zygotes through day 3 cleavage stage embryos and blastocyst stage embryos.	1 year 8 weeks after opening	2–8°C
Vit Kit-Thaw	90137-SO	TS, 4 × 2 mL DS, 1 × 2 mL WS, 1 × 2 mL	For use with oocytes (MII), pronuclear (PN) zygotes through day 3 cleavage stage embryos and blastocyst stage embryos.	1 year 8 weeks after opening	2–8°C
Sperm Washing Medium	9983	12 × 12 mL 100 mL	Ready-to-use outside the incubator. Contains 5 mg/mL HSA. Add preferred antibiotics.	2 years 8 weeks after opening	2–8°C
Arctic Sperm Cryopreservation Medium	90170	12 × 5 mL	Contains antioxidants, such as hypotaurine and ascorbic acid, is dual buffered with HEPES and MOPS, and contains 20 mg/mL Human Serum Albumin.	18 months 7 days after opening	2–8°C
Hyaluronidase	90101	5 x 1 mL	Contains 5 mg/mL Human Serum Albumin and 10 µg/mL Gentamicin sulfate in a HEPES-buffed HTF medium.	1 year Single-use	2–8°C
7% Polyvinylpyrrolidone (PVP) Solution with HSA	90121	5 × 0.5 mL	PVP 7% has relatively less viscosity than PVP 10%, contains 5 mg/mL Human Serum Albumin and is reconstituted in isotonic HEPES-buffered HTF medium.	183 days Single-use	2–8°C
10% Polyvinylpyrrolidone (PVP) Solution with HSA	90123	5 × 0.5 mL	PVP 10% has relatively more viscosity than PVP 7%, contains 5 mg/mL Human Serum Albumin and is reconstituted in isotonic HEPES-buffered HTF medium.	183 days Single-use	2–8°C

*From date of manufacture

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