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## Flush

### Complete Flush Solution

Complete Flush Solution is a modification of Holding Plus medium. It is not a substitute for Holding Plus, because many of the labile components have been removed to make the solution stable between 15°C and 30°C.

It is similar, however, because the same buffering system, energy sources, and membrane stabilizers have been retained. Complete Flush Solution and Holding Plus both use HEPES buffering at a concentration that is suited for an air atmosphere and not a CO<sub>2</sub> atmosphere. The surfactant properties of proteins are provided by 1mg/ml poly vinyl alcohol (PV-OH). Because proteins also act as heavy metal chelators, Bioniche has added other components necessary to fulfill this function.

This medium is packaged in 1 and 2 liter EVA infusion style bags.

These bags have a total of three ports: one port for filling and two extraction ports (a needle septum port and a spike port).

Shelf life is 2 years from date of manufacture.

**Recommended storage is 15-30° C.**

**1 liter Product number: EVM014**  
**2 liter Product number: EVM015**

## Holding

### Holding Plus

Holding Plus is the first bovine embryo transfer holding medium based on a formula adapted from a proven embryo culture medium. Holding Plus is designed to support optimal embryo survival in air at room temperature and provides essential amino acids, growth factors, enzymes, energy substrates and antibiotics.

Holding Plus is not an appropriate medium for long-term culture of bovine embryos in a CO<sub>2</sub> incubator.

This medium is packaged in EVA pouches with a needle septum port and will become available in a disposable tube.

Shelf life is 18 months from date of manufacture, provided sterile technique is used.

**Recommended storage is 2-8°C.**

**50ml Product number: EVM024**  
**20ml Product number: EVM224**  
**8 ml Product number: EVM824**

## Freeze

### Ethylene Glycol Freeze Plus

The success and convenience of freezing embryos in ethylene glycol for direct transfer brought about a dramatic change in the ET industry. Because ethylene glycol moves across cell membranes more readily than glycerol, embryos can be transferred directly into recipients without removing the embryo from the straw, eliminating time-consuming rehydration steps.

Time spent preparing embryos for freezing can also be greatly reduced.

Ethylene Glycol Freeze Plus with 0.1M sucrose and Ethylene Glycol Freeze Plus without Sucrose both contain 1.5M ethylene glycol and 0.4% BSA.

These media are packaged in EVA pouches with a needle septum port and will become available in a disposable tube.

Shelf life is 18 months from date of manufacture, provided sterile technique is used.

**Recommended storage is 2-8°C.**

### Ethylene Glycol Freeze Plus with 0.1M Sucrose

**50ml Product number: EVM034**  
**20ml Product number: EVM234**  
**8 ml Product number: EVM834**

### Ethylene Glycol Freeze Plus without Sucrose

**50ml Product number: EVM035**  
**20ml Product number: EVM235**  
**8 ml Product number: EVM835**



### Freeze Plus with 10% Glycerol

Freeze Plus is a complete glycerol freezing solution packaged for immediate use. It is a DMPBS-based solution containing 10% cell culture grade glycerol, 0.4% BSA and 0.1M sucrose. The addition of a low concentration of sucrose serves to protect blastomere membranes from osmotic stress during cryoprotectant equilibration.

This media is packaged in EVA pouches with a needle septum port and will become available in a disposable tube.

Shelf life is 18 months from date of manufacture, provided sterile technique is used.

**Recommended storage is 2-8°C.**

**50ml Product number: EVM032**  
**20ml Product number: EVM232**  
**8 ml Product number: EVM832**



## Thaw

### Thaw 1,2,3 Plus Kit

This kit contains three solutions (Thaw 1 Plus, Thaw 2 Plus, and Thaw 3 Plus), for three-step embryo rehydration. Each solution is a complete mixture containing 0.4% BSA and decreasing concentrations of glycerol (5%, 2.5%, 0.0%) and sucrose (0.5M, 0.5M, 0.6M). This combination of glycerol, sucrose, and BSA has proven to work very well across various freezing protocols. It is ideal for embryos with compromised zonae and provides an excellent safety factor on cold days when glycerol diffusion from embryos is slower or on hot days when higher sucrose concentrations may be detrimental.

These media are packaged in EVA pouches with a needle septum port and will become available in a disposable tube.

Shelf life is 18 months from date of manufacture, provided sterile technique is used.

**Recommended storage is 2-8°C.**

**3 x 50ml Product number: EVM048**  
**3 x 20ml Product number: EVM248**  
**3 x 8ml Product number: EVM848**



### One-Step Thaw Plus

The use of a 1M sucrose solution for the rapid one-step rehydration of embryos is convenient for thawing embryos cryopreserved in glycerol solution. This medium combines the convenience of a 1M thawing solution with the exceptional quality of ViGRO® Plus media.

This medium is packaged in EVA pouches with a needle septum port and will become available in a disposable tube.

Shelf life is 18 months from date of manufacture, provided sterile technique is used.

**Recommended storage is 2-8°C.**

**50ml Product number: EVM047**  
**20ml Product number: EVM247**  
**8 ml Product number: EVM847**



### Trypsin Wash

Trypsin Wash is formulated to maintain maximum cell viability while gently removing adherent cellular material from the zona pellucida. It is a 1:250 solution of Hank's Balanced Salts Solution (HBSS) consisting of a unique blend of serine proteases derived from porcine pancreas. Trypsin Wash is highly purified, tested to be virus and mycoplasma-free, and 0.22µ filtered. Trypsin Wash meets IETS Manual guidelines (Chapter 6, third edition). This medium is packaged in biofreeze vials. Shelf life is 9 months from date of manufacture.

**This product must be stored frozen.**  
**5ml Product number: EVM050**



## Additional Media

### Splitting Plus

Embryo splitting is the most effective and rapid method of increasing the number of offspring from an ET program. This medium is designed for safe and rapid splitting or biopsy without the need for a holding pipette. It contains no protein and consequently allows proteins in the zona pellucida to form an electrostatic attraction to the bottom of the tissue culture dish; this holds the embryo in place during the splitting procedure. Used in conjunction with Bioniche Animal Health's Twinner System, it provides the ET practitioner with a cost-effective method for safe, precise splitting or biopsy procedures.

This medium is packaged in EVA pouches with a needle septum port. Shelf life is 2 years from date of manufacture, provided sterile technique is used.



**Recommended storage is 15-30°C.**  
**50ml Product number: EVM062**

### Retrieval Supplement PF

This is a protein-free (PF) solution which provides the "non-stick" characteristics of serum or BSA without the viral, antibody or DNA contamination problems that are associated with the use of animal products. This supplement has many applications as a replacement for FCS or BSA in flushing solutions. Its primary use, however, is in embryo splitting and sexing. Following bisection or biopsy, a few microliters of this supplement neutralizes the electrostatic attraction of the culture dish and allows for easy recovery of demi-embryos or the risk-free transfer of biopsies to amplification capillaries. Contaminating DNA can produce false positives when using PCR diagnostic testing. This supplement eliminates the possibility of introducing bovine DNA, which is often found in FCS or BSA.

This medium is packaged in EVA pouches with a needle septum port. Shelf life is 2 years from date of manufacture, provided sterile technique is used.



**Recommended storage is 15-30°C.**  
**50ml Product number: EVM064**

### Rinsing Solution

Rinsing Solution is the same formula as Complete Flush Solution, except that it does not contain any surfactant. Because surfactants tend to foam when agitated, Rinsing Solution is designed for rinsing embryo collection filters at the end of the collection.

If media containing surfactants are used for filter washing, embryo searching may prove difficult until the bubbles clear. Although embryos usually do not stick to filter membranes, it is good practice to thoroughly wash the filter.

This medium is packaged in 1 liter EVA infusion style bags. These bags have a total of three ports: one port for filling and two extraction ports (a needle septum port and a spike port). Shelf life is 2 years from date of manufacture.



**Recommended storage is 15-30°C.**  
**1 liter Product number: EVM100**

### TL HEPES

Our TL HEPES is used for rinsing oocytes in certain steps of IVF protocols. TL HEPES is produced both with and without 0.3% BSA and is packaged in sterile 500ml high-density PETG bottles with screw closure. A heat shrink band around the closure and neck provide a tamper-proof seal.

Shelf life for both versions of TL HEPES is 1 year from date of manufacture, provided the neck seal is unbroken.

**Recommended storage for TL HEPES (without BSA) is 15-30°C.**

**TL HEPES 500ml Product number: IVF022**

**Recommended storage for TL HEPES with BSA is 2-8°C.**

**TL HEPES with BSA 500ml Product number: IVF032**





# SYNGRO® Media

## Holding

**SYNGRO®** Holding is a solution designed to provide an environment closely resembling that of the uterus for maintaining embryos in an air atmosphere at temperatures from 18-25°C for up to 9 hours.

**Recommended use:** Embryos should be recovered from the flushing solution and transferred to a covered petri dish containing **SYNGRO®** Holding. Embryos can be held at room temperature for up to 9 hours or up to 20 hours at 4°C. Embryos can be transferred using a straw containing **SYNGRO®** Holding.

**Recommended storage:** 15-30°C. DOES NOT REQUIRE REFRIGERATION. This product does not contain materials of animal origin.

50ml Product Number: ESM024    20ml Product number: ESM224    8ml Product number: ESM824 (6 vials / box)

All **SYNGRO®** brand media contain no materials of animal origin and do not require refrigeration. This provides practical shipping and handling advantages, while reducing the risk potential of inadvertent contamination from viruses and bacteria. Bioniche developed the **SYNGRO®** technology in anticipation of stricter government restrictions in the international movement of embryos. Look for the special logo shown below on all **SYNGRO®** products as your assurance of animal-origin free formulas to reduce the chances of disease transmission.

Non-refrigeration plays an important role in reducing the total cost of your product orders as this feature provides the opportunity to use your freight company's "best available rates" for shipping, rather than second-day air for refrigerated products. As well, **SYNGRO®** products may be used immediately without waiting for them to warm to room temperature each time they are needed.



## Equine Specialty Media

### Equine Uterine Lavage Solution

This lavage medium is appropriate for use in mares with post-foaling, bacterial, mycotic, or placental endometritis. It is also useful in pre- or post-breeding and pre- or post-insemination lavage. An iso-osmotic electrolyte solution, it is buffered to a physiological pH of 7.1 and is made from water purified by reverse osmosis to 18 megohms resistance. It contains PVA as a surfactant and is filtered to 0.2 microns. Packaged in 2-liter, sterile, EVA infusion-style bags, it can be stored at room temperature. Shelf life is one year from the date of manufacture.

**Recommended storage is 15-30°C.**  
**2 liter bags**  
**Product number: EQU100**



### Equine Vitrification Kit

This kit was developed by the Equine Reproduction Laboratory at Colorado State University and is now commercially available for the vitrification of equine embryos without the need for additional, expensive equipment.

Each kit comes with complete, detailed directions and contains one 8 ml vial of each of the following media: VS1 (Vitrification Solution 1), VS2, VS3, & Diluent.

**Recommended storage is 2-8°C.**  
**4 x 8ml vials**  
**Product number: EQU030**



### General Information

ViGRO® media comprise a unique group of solutions designed specifically for the exacting needs of embryo transfer in both commercial and research applications. These media are unique because they embrace modern cell culture technology and because they are made to quality control standards that exceed regulatory requirements.

Production is carried out in FDA-inspected, particle-free, pressurized clean rooms where GMP (FDA's Pharmaceutical Good Manufacturing Practice) is the minimum standard. Only ultra pure chemicals are used and all components are fully traceable. Each medium is serially filtered. The Complete Flush Solution and Rinsing Solution are 0.2µm filtered. Holding Plus, Freeze Plus, Ethylene Glycol Freeze Plus, Thaw 1,2,3 Plus Kits, One-Step Thaw Plus, Retrieval Supplement PF, and Splitting Plus are 0.1µm filtered. Even mycoplasma are removed by this micro-filtering technique before media are dispensed into gamma sterilized packaging.

All batches of ViGRO® media are tested to ensure correct levels of sodium, calcium, magnesium, potassium, chloride, and where needed, proteins. Osmolality must be between 280 mOsm and 295 mOsm and pH must be between 7.3 and 7.5. Recommended storage of ViGRO® media is between 2-8°C (except where noted). The entire ViGRO® product range is complete and ready to use; no further additions are required. The ultra filtering process used during manufacturing ensures that no additional filtering is required before use. These quality-oriented features have made ViGRO® media a benchmark for embryo transfer solutions throughout the world.

### Reagent Quality

The process begins with highly purified certified chemicals. Particular attention is paid to heavy metal contamination and the level of endotoxins in all of our reagents. Our protein products originate from animals that were inspected both pre- and post-mortem, certified free of mycoplasma and seven major virus groups, and approved for use by the USDA with both domestic and export embryos. We then purchase large batches for uniformity of product performance. Full certification is available upon request.

### Water Quality

Water purification starts with pre-conditioning involving a sodium ion exchange system for divalent and multivalent ions, followed by 5µm filtration, then activated carbon absorption filtration to remove chlorine, particulate matter, and other organic compounds. The pre-conditioned water then travels to a reverse osmosis (RO) unit where 98% of remaining ions, microbial impurities and most of the remaining organics are removed. Storage of ultra-pure water in glassware and non-polyethylene containers may cause an increase in the amount of heavy metals and/or total organic carbons in water in only a few days' time. Ultra-pure water leaches the impurities out of the walls of containers. The RO water used in ViGRO® media is stored in rigid polyethylene reservoirs that will not leach impurities.

The RO water then feeds from the reservoir to a second activated carbon absorption filter to insure removal of organics and chlorine. From the carbon filter, the water passes through a layer of macroreticular resin to remove colloids and then passes through a two-bed cation and anion resin which removes the majority of ionic components. Finally it passes through an ultra-pure mixed-bed cartridge, which removes all remaining ions yielding up to 18.3 mega-ohm water, which is essentially free of ionic contaminants.

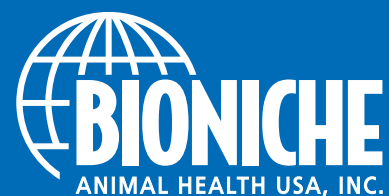
Any remaining organics are removed by using a high-efficiency synthetic carbon filter. A final 0.2µm cross-flow filter is incorporated in the dispenser for even greater assurance of freedom from contamination. The water is now as pure as is technically possible and pyrogen-free.

### Laboratory Procedure

The media production facility is a positive pressure, HEPA filtered room equipped with class 100 isolation areas, with staff utilizing clean room dress and procedures. Serial filtration occurs in the class 100 isolation areas, with all media (except Complete Flush Solution, Rinsing Solution, and Trypsin) passing through a final ultra-fine filter at the point of dispensing. Following packaging under an inert atmosphere to increase shelf-life, product samples are forwarded to independent analytical laboratories for analysis and quality control testing. Certificates of analysis are available for each batch of ViGRO® media.

### Packaging

EVA, utilized in our media packaging, is superior to traditional plastic film because it is manufactured specifically for packaging pharmaceutical products. EVA has an ultra low level of extractibles and contains no plasticizers. The full transparency of EVA is a major advantage for visual inspection of both the solution and the empty bag.



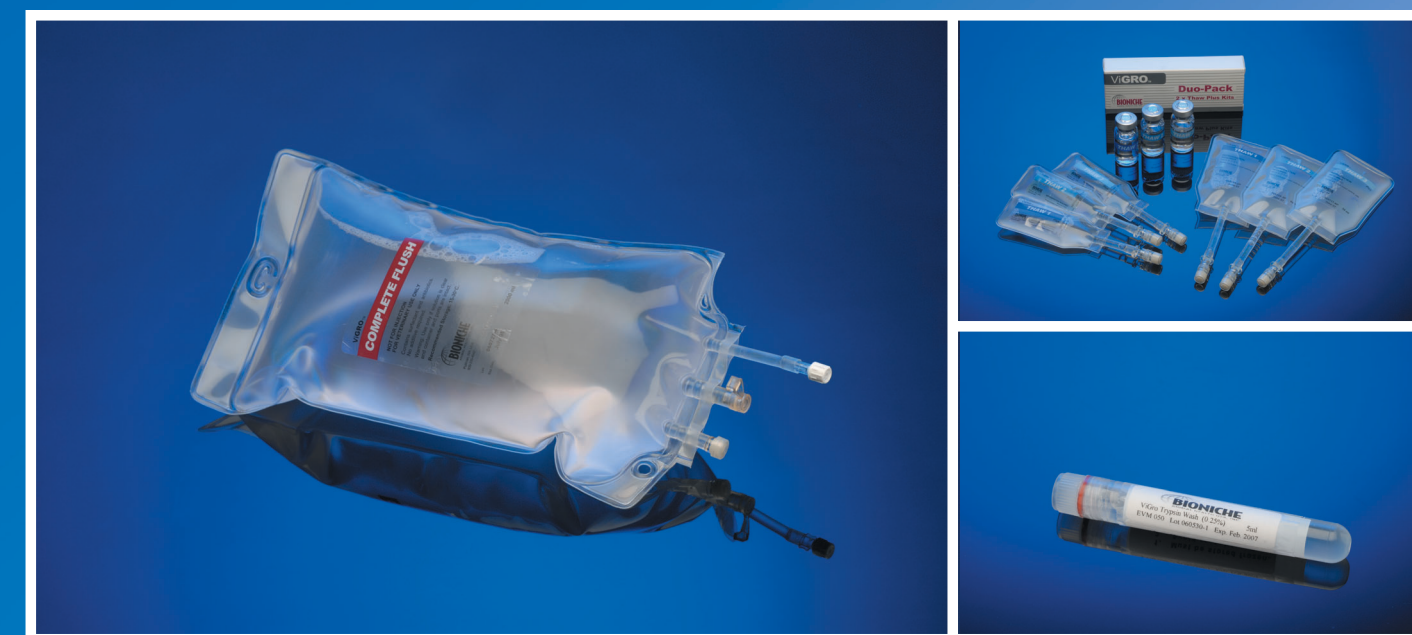
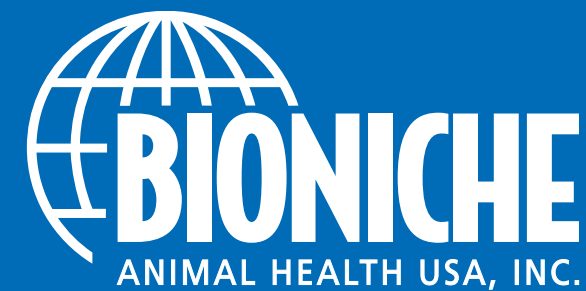
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## Embryo Transfer Media