

CULTREX[®] Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Cultrex[®] High Protein Concentration Basement Membrane Extract (HC20+[™]) *PathClear[®]

Catalog #: 3444-005-02

Size: 5 ml

Description: Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between endothelial, epithelial, muscle, or neuronal cells and their adjacent stroma. Basement membranes are degraded and regenerated during development and wound repair. They not only support cells and cell layers, but they also play an essential role in tissue organization that affects cell adhesion, migration, proliferation, and differentiation. Basement membranes provide major barriers to invasion by metastatic tumor cells. Cultrex Basement Membrane Extract is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumors. The extract gels at 37°C to form a reconstituted basement membrane. The major components of the Basement Membrane Extract (BME) include laminin, collagen IV, entactin, and heparin sulfate proteoglycan.

Cultrex[®] HC20+[™] PathClear[®] was developed for use in *in vivo* applications where higher protein concentrations facilitate faster gelling times, increased gel strength, and elevated levels of tumor augmentation. Cultrex[®] HC20+[™] PathClear[®] is pathogen-free*, has the advantage of lot-to-lot consistency and controlled protein concentrations to support *in vivo* angiogenesis assays and tumorigenicity assays.

Specifications:

Concentration: ≥ 20 mg/ml

Source: Murine Engelbreth-Holm-Swarm (EHS) tumor

Storage Buffer: Dulbecco's Modified Eagle's medium containing 10 µg/ml gentamycin sulfate and no phenol red.

*PathClear[®]: Negative by PCR test for: mycoplasma, 17 bacterial and virus strains typically included in mouse antibody production (MAP) testing, plus 13 additional murine infectious agents including LDEV, for a total of 31 organisms and viruses.

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TREVIGEN[®]

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Storage/Stability: Product is stable for a minimum of 3 months from date of shipment when stored at $-20\text{ }^{\circ}\text{C}$ in a manual defrost freezer. **For optimal stability, store at $-80\text{ }^{\circ}\text{C}$. Keep Frozen; repeated freeze-thaws will destroy product integrity.**

Material Qualification:

Gelling: PathClear[®] BME gels in less than 30 minutes at $37\text{ }^{\circ}\text{C}$, and maintains the gelled form in culture medium for a minimum of 14 days at $37\text{ }^{\circ}\text{C}$.

Functional Assay:

• Tube formation: BME promotes differentiation of a mouse endothelial cell line derived from axillary lymph node (SVEC4-10) into capillary-like structures.

Sterility Testing:

- No bacterial or fungal growth detected after incubation at 37°C for 14 days following USP XXIV Chapter 71 sterility test.
- Negative by PCR test for mycoplasma, 17 bacterial and virus strains typically included in mouse antibody production (MAP) testing, plus 13 additional murine infectious agents, for a total of 31 organisms and viruses.
- Endotoxin concentrations $\leq 20\text{ EU/ml}$ by LAL assay.

HC20+ PathClear[®] Thawing/Handling Instructions:

Thaw HC20+ PathClear[®] at $2\text{-}8^{\circ}\text{C}$ overnight. Refrigerator temperatures may vary; therefore, thaw extract on ice in a refrigerator. HC20+ gels very quickly at room temperature; for best results, keep on ice. It may also be necessary to pre-chill pipette tips, tubes, plates, or other objects that may come in contact with the extract.

Promotion of Tumor Growth and Assays for Tumorigenicity

Tumor Cell Culture:

Tumor cells should be cultured as recommended by supplier. For tumorigenicity assays, 100,000 to 1,000,000 cells are generally injected per mouse; plan ahead to make sure that a sufficient number of cells is cultured prior to assay. There is always loss of cells when filling syringes, so plan to culture twice as many cells as needed.

Tips for Selecting/Handling Mice:

- Mice do not need to be anesthetized for this procedure.
- Females often fight less than males and are usually better to use.
- Nude mice are usually calm and will not move too much if you give them the lid of the cage to hold on to.
- Mouse restrainers may be used as needed.
- Use nude mice for human cell lines.

Procedure:

1. Thaw HC20+ PathClear[®] as indicated (above).
2. Harvest tumor cells by standard method, and centrifuge at $250\text{ } \times\text{ } g$ to pellet.
3. Remove supernatant, and resuspend cells in medium (e.g. DMEM) minus serum.
4. Count the cells, and transfer two times the exact number of cells needed for the assay to another tube. Reserve one tube per mouse.
5. Centrifuge cells for 5 minutes at $250\text{ } \times\text{ } g$.
6. Remove supernatant, and put cell pellet on ice.
7. Resuspend the loosened cell pellet in 0.5 ml of cold HC20+ per tube for each mouse. Mix well, and keep on ice.

8. Inject 0.5 ml of cold cell suspension, subcutaneously, using a 25 gauge needle in a 1 ml syringe. See Tips for Selecting/Handling Mice (page 2). After injection, leave the needle in for about 20 seconds to allow HC20+ to gel, and then rotate the needle when removing it from the injection site to reduce leakage (there will be a big bump visible and some leakage of liquid is possible).
9. Tumors may begin to grow as early as 7 days depending on the number of cells injected. Check mice weekly.

Other Applications

HC20+ may also be substituted for BME for all known assays, but the higher protein concentration often results in faster gelling and firmer gels. Please see our product data sheets for our standard Basement Membrane Extract for additional protocols.

Related Products:

Catalog#	Description	Size
3455-024-K	Cultrex® 24 Well BME Cell Invasion Assay	24 inserts
3480-024-K	CultreCoat® 24 Well BME-Coated Cell Invasion Assay	24 inserts
3456-024-K	Cultrex® 24 Well Laminin I Cell Invasion Assay	24 inserts
3457-024-K	Cultrex® 24 Well Collagen I Cell Invasion Assay	24 inserts
3458-024-K	Cultrex® 24 Well Collagen IV Cell Invasion Assay	24 inserts
3455-096-K	Cultrex® 96 well BME Cell Invasion Assay	96 samples
3465-024-K	Cultrex® 24 well Migration Cell Assay	96 samples
3465-096-K	Cultrex® 96 well Migration Cell Assay	96 samples
3456-096-K	Cultrex® Laminin I Cell Invasion Assay	96 samples
3457-096-K	Cultrex® Collagen I Cell Invasion Assay	96 samples
3458-096-K	Cultrex® Collagen IV Cell Invasion Assay	96 samples
3490-096-K	CultreCoat® BME 96 Well Cell Adhesion Assay	96 samples
3496-096-K	CultreCoat® 96 Well Adhesion Protein Array	96 samples
3450-048-SK	Cultrex® Directed In Vivo Angiogenesis Assay (DIVAA™) Starter	48 samples
3450-048-K	Cultrex® DIVAA™ Kit	48 samples
3450-048-IK	Cultrex® DIVAA™ Inhibition Kit	48 samples

Accessories:

Catalog#	Description	Size
3400-010-02	Cultrex® Mouse Laminin I, PathClear®	1 ml
3400-010-01	Cultrex® Mouse Laminin I	1 mg
3440-100-01	Cultrex® Rat Collagen I	100 mg
3410-010-01	Cultrex® Mouse Collagen IV	1 mg
3420-001-01	Cultrex® Human Fibronectin, PathClear®	1 mg
3416-001-01	Cultrex® Bovine Fibronectin, NZHD*	1 mg
3421-001-01	Cultrex® Human Vitronectin, PathClear®	50 µg
3417-001-01	Cultrex® Bovine Vitronectin, NZHD	50 µg
3438-100-01	Cultrex® Poly-L-Lysine	100 ml
3439-100-01	Cultrex® Ploy-D-Lysine	100 ml
3445-048-01	Cultrex® 3-D Culture Matrix™ BME	15 ml
3446-005-01	Cultrex® 3-D Culture Matrix™ Laminin I	5 ml
3447-020-01	Cultrex® 3-D Culture Matrix™ Collagen I	100 mg
3430-005-01	Cultrex® BME with Phenol Red	5 ml
3432-005-01	Cultrex® BME without Phenol Red	5 ml
3431-005-01	Cultrex® BME with Phenol Red; Reduced Growth Factor	5 ml
3433-005-01	Cultrex® BME; no Phenol Red; Reduced Growth Factor	5 ml

*New Zealand Herd Derived

Catalog#	Description	Size
3415-001-02	Cultrex [®] Human BME, PathClear [®]	1 ml
3430-005-02	Cultrex [®] BME with Phenol Red, PathClear [®]	5 ml
3431-005-02	Cultrex [®] BME with Phenol Red, Growth Factor Reduced PathClear [®]	5 ml
3432-005-02	Cultrex [®] BME, PathClear [®]	5 ml
3433-005-02	Cultrex [®] BME, Growth Factor Reduced, PathClear [®]	5 ml
3437-100-K	Cultrex [®] Cell Staining Kit	100 ml
3450-048-05	CellSperse [™]	15 ml

References:

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2. Fridman, R., G. Giaccone, T. Kanemoto, G. Martin, A. Gazdar, and J. Mulshine. 1990. Reconstituted basement membrane (matrigel) and laminin can enhance the tumorigenicity and the drug resistance of small cell lung cancer cell lines. *Proc. Natl. Acad. Sci. USA* **87**:6698-6702.
3. Fridman, R., M. Kibbey, L., Royce, M. Zain, T. Sweeney, D. Jicha, J. Yannelli, G. Martin, and H. Kleinman. 1991. Enhanced tumor growth of both primary and established human and murine tumor cells in athymic mice after coinjection with matrigel. *J. Natl. Cancer Inst.* **83**:769-774.
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5. Kubota, Y., H. Kleinman, G. Martin, and T. Lawley. 1988. Role of laminin and basement membrane proteins in the morphological differentiation of human endothelial cells in capillary-like structures. *J. Cell Biol.* **107**:1589-1598.
6. Ponce, M., M. Nomizu, M. Delgado, Y. Kuratomi, M. Hoffman, S. Powell, Y. Yamada, H. Kleinman, and K. Malinda. 1999. Identification of endothelial cell binding sites on the laminin γ 1 chain. *Circ. Res.* **84**:688-694.
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10. Arnaoutova, I., J. George, H.K. Kleinman, and G. Benton. 2009. The endothelial cell tube formation assay on basement membrane turns 20: state of the science and the art. *Angiogenesis* doi:10.1007/s10456-009-9146-4
11. U.S. Patent 4,829,000
12. U.S. Patent 5,158,874

This product is made and marketed under patent license from the United States Public Health Service. Ref. U.S. Patent 4,829,000 issued May 9, 1989 and U.S. Patent 5,158,874 issued October 27, 1992, all entitled Reconstituted Membrane Complex with Biological Activity.



**High Protein
Concentration Basement
Membrane Extract
(HC20+™) PathClear[®]**
 Cat#: 3444-005-02
 Storage: ≤ -20 °C
 (Manual Defrost Freezer)
 1-800-873-8443

Lot Specific Data:

Protein Concentration:
 Endotoxin (LAL):