

ASCC CELL CULTURE MEDIUM LICENSING PROPOSAL

MEDIUM NAME:

APEL HESC Differentiation Medium

SUMMARY:

APEL (Albumin Polyvinylalcohol Essential Lipids) medium is a chemically defined serum free cell culture medium that provides a robust platform for the differentiation of human Embryonic Stem cells (HESCs). Unlike alternative culture mediums, APEL does not contain serum or serum derived components that are undefined and subject to lot-to-lot variability, which can influence the outcome of differentiation experiments and modify the effects of added growth factors. APEL has been used in combination with multiple combinations of growth factor cocktails to differentiate HESCs into cells of all 3 germ layers as determined by marker expression.

LICENSING TERMS:

Type: Exclusive

DETAILED DESCRIPTION:

APEL HESC Differentiation Medium is a novel defined, animal free cell culture medium intended to be used in combination with recombinant growth factors for the differentiation of HESCs. Due to the absence of undefined components, APEL provides a robust platform for the optimisation of HESC differentiation. Unlike alternative culture mediums, APEL does not contain serum or serum derived components that are undefined and subject to lot-to-lot variability, which can influence the outcome of differentiation experiments and modify the effects of added growth factors. By removing undefined components, APEL provides researchers with a superior basis to analyse the effects of exogenous growth factors upon HESC differentiation. Furthermore, the removal of heterogeneous components also significantly improves reproducibility and enables more accurate comparisons of results to be made. Being animal component free, APEL may also be valuable for the future generation of pathogen free differentiated cells for clinical use.

APEL HESC Differentiation Medium has been validated on 6 different HESC lines and sublines that have been cultured using enzymatic expansion. Moreover, the potential of HESC to differentiate into cells representing all 3 germ layers has been demonstrated via marker expression.

For the improved differentiation of HESCs, APEL HESC Differentiation Medium can be used in combination with the ASCC developed SPIN Embryoid Body (EB) method

(see separate proposal). However it should be noted that APEL is also suitable for use with HESC monolayer differentiation cultures.

CELL TYPES & SPECIES CULTURED:

Human Embryonic Stem Cells

FORMULATION:

APEL HESC Differentiation Medium is manufactured from components that are commercially available. Whilst formulation details are provided below, please refer to the attached reference for complete details.

Media Component (Final Concentration)	Stock Solution	Volume per 200 mL APEL
1 X Iscove's modified Dulbecco's medium (IMDM)	1 X	86.2
1 X Ham's F-12 nutrient mixture	1 X	86.2
Albucult (rh Albumin) (5 mg ml ⁻¹)	100 mg mL ⁻¹ (10%)	10 mL
Polyvinylalcohol (PVA)	5 or 10%	1 or 2 ml
Linoleic acid (100 ng ml ⁻¹)	10,000 X	20 µl
Linolenic acid (100 ng ml ⁻¹)	10,000 X	20 µl
SyntheChol (synthetic cholesterol) (2.2 mg ml ⁻¹)	7,200 x	28 µl
α-Monothioglycerol (α-MTG) (3.9 ml per 100 ml)	13 ml in 1 ml IMDM	600 µl
rh Insulin-transferrin-selenium-ethanolamine solution	100 x	2 ml
Protein-free hybridoma mixture II (PFHMII) (5%)	1 x	10 ml
Ascorbic acid 2 phosphate (50 mg ml ⁻¹)	5mg ml ⁻¹	2 ml
GlutamaxI (L-alanyl-L-glutamine) (2 mM) (100_) 2 ml	200 mM	2 ml
Penicillin/streptomycin (50 U Pen G/50 mg streptomycin sulfate)	200 X	1 ml

PREPARATION:

For preparation of 200 ml APEL Medium.

Timing:

- Preparation of PVA stock solutions requires 2–4 d.
- Assembly of APEL medium from its components requires approx. 1 h

1. Prepare a 5 or 10% (wt/vol) stock solution of PVA. Add 5 or 10 g of PVA to 100 ml dH₂O and leave to dissolve at 4°C for 2–4 d. Alternatively, after leaving the mixture of PVA and water at 4°C for a few hours to allow the PVA to become 'wet', the solution can be placed in a 37°C water bath overnight to facilitate solubilization. As an option, the PVA solution can be prefiltered through a 0.45-µm filter unit to reduce viscosity before use in the APEL medium. In our laboratory, both 5 and 10% PVA stock solutions are used successfully without the need for the prefiltration step.

Note: The PVA stock solutions are stable at 4 °C for at least 3 months.

2. Make a PVA–lipids mixture by adding ~20 ml IMDM and 20 ml F-12 nutrient mixture to a 50-ml tube, followed by the required volumes of PVA stock, SyntheChol, linoleic and linolenic acids for 200 ml APEL as described above. Shake vigorously to mix the contents of the tube.

CRITICAL STEP This mixing step prevents the PVA from forming insoluble droplets in the medium.

3. Add the remaining media components (i.e., the balance of the required volumes of IMDM and F-12, AlbuCult, α -MTG, 100X rhITS-Eth, PFHMII, ascorbic acid, Glutamax I, penicillin and streptomycin) directly to the upper chamber of a 0.22- μ m SteriCup filtration unit. Add the PVA–lipids mixture from Step 2 and filter the medium. As an option, a glass 0.45- μ m filter disc can be placed on the 0.22- μ m filter surface of the upper chamber of the SteriCup unit to facilitate filtration.

STORAGE CONDITIONS & STABILITY:

APEL HESC Differentiation Medium can be stored for up to 2 weeks at 4°C.

COMPETITIVE ADVANTAGES:

1. Permits the improved formation of EBs
2. Superior reproducibility of EB formation & differentiation
3. Neutral medium that does not influence HESC differentiation
4. Provides a superior basis for the determination of the effect of exogenous growth factors upon HESC differentiation
5. Minimal batch-to-batch variability
6. Could be produced at GMP quality for the manufacture of differentiated cells for cell therapy
7. Validated on multiple HESC lines

PUBLICATIONS:

1. Ng E.S. et al (2008). Nature Protocols Vol. 3 No. 5, 768 (Attached)

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