Accumax™ Dissociation Reagent for the Serial Passaging of Human Embryonic Stem Cells

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Abstract

Currently, there are two widely accepted methods for dissociating human embryonic stem cells (hESCs) for passaging: (1) manual passaging, which is the gentlest way to dissociate hESCs, but is also very tedious and time-consuming, and (2) enzymatic passaging, which is much easier than manual passaging and can be applied to higher throughput processing of hESCs, but is associated with concerns of cell health and genomic stability[1]. For enzymatic passaging, many researchers use a type IV collagenase derived from the bacterium *Clostridium histolyticum*. Here we show that the product, Accumax reagent (Cat. No. SCR006) is suitable for passaging human embryonic stem cells in cultures using feeder layers. After cells went through fifteen consecutive serial passages using Accumax reagent, they were shown to maintain pluripotency by morphology and expression of appropriate markers; they also maintained a normal karyotype. In addition, it was noted that there were several advantages to using Accumax reagent versus the type IV collagenase derived from *Clostridium histolyticum*.

Methods

General hESC culture

H9 human ES cells were maintained on CF1 mouse embryonic fibroblasts (Cat. No. PMEF-CF) seeded at a density of 40,000 fibroblasts/cm^2 on tissue culture plates coated with growth factor-reduced Matrigel® EHS matrix (BD Biosciences). hESCs were maintained in a medium containing 20 ng/mL basic FGF (Cat No. GF003); media was replaced every 1–2 days. Cultures were passaged every 6 or 7 days regardless of the dissociation method used (see below).

Dissociating hESCs for passaging with Accumax reagent

Cells growing in adherent culture as described above were rinsed once with Dulbecco's PBS (Cat. No. BSS-1006), then overlaid with Accumax reagent at a volume of approximately 0.05-0.1 mL/cm^2. Cultures were incubated in Accumax reagent for exactly 5 minutes at room temperature, then cells were quickly dislodged from the culture surface (using a serological pipet) and the suspension removed to a tube containing culture media at 5 times the volume of Accumax reagent used. Cells were briefly agitated by gentle pipetting, and were then centrifuged at 75 x g for 5 minutes. After the supernatant was aspirated, cells were gently resuspended in culture media and split at a ratio of 1:3.

Dissociating hESCs for passaging with collagenase

As a control for the use of Accumax reagent, separate cultures of hESCs were maintained under identical conditions, except that they were dissociated for passaging using the type IV collagenase derived from Clostridium histolyticum. Cultures were rinsed once with Dulbecco's PBS, then overlaid with approximately 0.1 mL/cm^2 of a 1 mg/mL collagenase solution. Cultures were incubated for 10 minutes at 37 °C, or until the edges of colonies began to separate from the culture surface. Cells were then scraped off of the culture surface and transferred to a tube containing culture media at 3 times the volume of collagenase used. Cells were briefly agitated by gentle pipetting, and were then centrifuged at 75 x g for 5 minutes. After the supernatant was aspirated, cells were gently resuspended in culture media and split at a ratio of 1:3.

Results

Parallel cultures of human ES cells were passaged using either Accumax reagent or type IV collagenase from Clostridium histolyticum, but were otherwise maintained under identical conditions. Different effects upon the cells were observed between the two enzymatic treatments. After 5 minutes of treatment with Accumax reagent, clusters of cells tended to show some fragmentation into individual cells at the edges of colonies, but otherwise clusters remained largely intact; in contrast, after treatment with collagenase for 10 minutes, clusters showed separation of the edge from the culture surface, but few individual cells were observed (not shown). The colonies resulting from Accumax reagent passaging were smaller and more numerous than those resulting from passaging with collagenase, indicating that Accumax reagent dissociates human ES cell clusters to smaller fragments than does collagenase. Finally, clusters of cells passaged by Accumax reagent treatment tended not to be as compact as those passaged by collagenase during the first few days of culture. However, by day 6 or 7 of culture, clusters passaged by the two treatments could not be distinguished from one another by morphology (Figure 1).

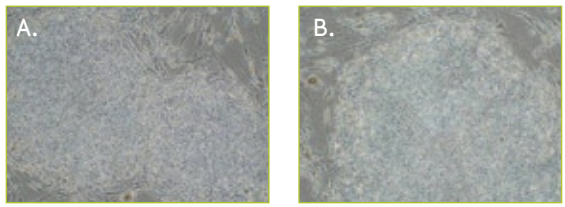

Figure 1. Morphologies of H9 human ES cells after 15 passages in either Accumax (A) or type IV collagenase. (B) are highly similar. H9 cells were grown on a CF1 mouse embryonic fibroblast feeder layer.

A.

After 15 consecutive passages by either one or the other treatment, cultures were analyzed for pluripotency and karyotypic stability. Pluripotency was examined by use of an alkaline phosphatase activity staining kit (Cat. No. SCR004), as shown in Figure 2, and by indirect immunofluorescent labeling with antibodies to OCT4 and SSEA-4 (Cat. Nos. MAB4401 and MAB4304), as shown in Figure 3; data is shown for cells treated with Accumax reagent, but similar results were observed for cultures passaged with type IV collagenase. As shown, cells passaged 15 times in Accumax reagent retained expression of pluripotent markers. In addition, a normal karyotype was maintained (data not shown).

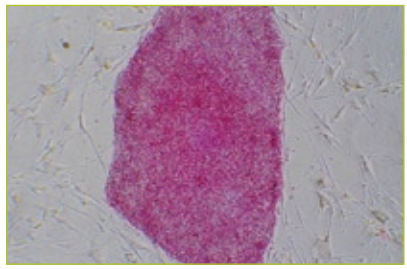

Figure 2. Alkaline Phosphatase expression in H9 human ES cells after 15 passages using Accumax reagent to dissociate the cells.

Discussion

This work demonstrates the utility of Accumax reagent for the dissociation and passaging of human ES cells. Like collagenase, Accumax reagent saves time and effort over manual passaging. Furthermore, cells passaged multiple times with Accumax reagent maintain expression of pluripotency markers as well as their normal karyotype.

There are some differences between Accumax reagent and collagenase that should be noted. In terms of ease of use of product, Accumax reagent is supplied as a sterile, ready-to-use 1X liquid, while collagenase is supplied as a powder that must be weighed out, put into solution and then filter-sterilized. Also, once thawed, Accumax reagent can be stored at 4 °C for up to two months (it can also be aliquotted and refrozen, if desired), whereas reconsituted collagenase must be immediately frozen, and subsequently thawed aliquots are generally discarded immediately after use. The differences between the two enzyme treatments in their effect upon human ES cells is also of note. By creating a greater number of smaller colonies (i.e., in comparison to collagenase), Accumax reagent is more useful for the expansion of cultures, including higher-throughput applications.

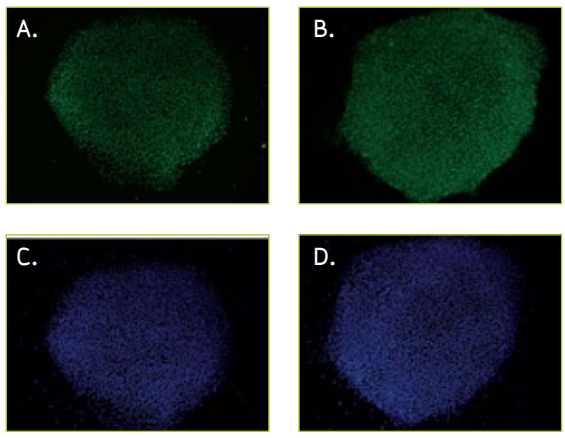

Figure 3. Expression of OCT4 (A) and SSEA-4 (B) pluripotency markers in H9 human ES cells after 15 passages using Accumax reagent to dissociate the cells. Corresponding DAPI images are shown in (C) and (D).

References

1. Hanson, C., Caisander, G. Human embryonic stem cells and chromosome stability. APMIS 2005;113: 751–5..

Description	Quantity*	Cat. No.
Accumax Reagent	100 mL	SCR006
Primary Mouse Embryonic Fibroblasts, Strain CF1, mytomycin C treated, passage 3	5 vials	PMEF-CF
DMEM/F12, with L-Glutamine	500 mL	DF-042-B
Basic FGF, Recombinant Human	50 µg	GF003
Alkaline Phosphatase Detection Kit	1 kit	SCR004
Oct-4 Monoclonal	100 µg	MAB4401
SSEA-4 Monoclonal	100 µg	MAB4304

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