Corgel® BioHydrogel (TS-NaHy)

Corgel® Cross-linking Procedure

CAUTION:
PLEASE READ ALL INSTRUCTIONS PRIOR TO USE,
AS THIS GEL SETS UP QUICKLY.

Product Description/Chemistry

Corgel® tyramine substituted sodium hyaluronate (TS-NaHy), is a novel hyaluronan-based hydrogel system formed by either *in vitro* or *in situ* cross-linking driven by peroxidase.

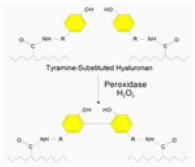

Illustration of the Cross-linking of TS-NaHy - not reflective of the cross-linking density

The use of this protocol will require optimization by the end user.

PLEASE REFRIGERATE UPON ARRIVAL

Reagents Provided

- Tyramine-Substituted Sodium Hyaluronate (TS-NaHy) (X% Substitution - see bottle label) - 250 mg powder. (**Storage**: 2 - 8° C)
- Horseradish Peroxidase (HRP) in PBS (10 U/mL) - 25 mL. Avoid prolonged exposure to light. **(Storage:** 2 - 8°C)
- Hydrogen Peroxide (H_2O_2) in PBS (1.0%) - 5 mL. Avoid prolonged exposure to light. **(Storage:** 2 - 8°C)

Expiration

- Lifecore has not established an expiration date for the product.

Procedures

All steps may be performed at ambient temperature, unless otherwise specified.

Caution - **If you require more than one concentration of hydrogel, either aliquot the TS-NaHy before hydration or hydrate to the highest desired concentration and then aliquot to dilute to the desired concentrations.**

1. Add HRP solution to hydrate TS-NaHy to the desired concentration. Use sufficient agitation and/or time to ensure that the solution is fully hydrated. When targeting a final gel concentration of TS-NaHy, take into account the amount of peroxide solution (step 2) to be added. Avoid direct sunlight.

Caution - **Aliquot the TS-NaHy solution for dilution and/or make additions (for example, adding cells or drugs) as required before proceeding to the next step.**

Notes:

- Gel concentrations below the lowest values and above the highest values in Table 1 may be generated, but the final hydrogel concentrations may not correlate due to expansion or contraction of the gel matrix.
- The gel consistency will vary from a soft gel to firm gel particulates, and from optically clear to slightly colored gel, depending on TS-NaHy substitution, gel concentration, H_2O_2 concentration, and additional components added.
- The gel will set up within seconds, however maximum strength will be achieved after 16 hours.
- Cells, bioactive agents, or other components may be added to the TS-NaHy solution during hydration, prior to H_2O_2 addition, or after gel formation. These additives may affect the physical and biological properties of the gel.

2. Crosslink the TS-NaHy solution by adding H_2O_2 solution of a desired concentration. **The recommended volume ratio of H_2O_2 solution to TS-NaHy solution is 1 to 25.** Mix during addition if required. The H_2O_2 reagent should be diluted with water to the desired concentration for cross-linking. Examples of the recommended H_2O_2 concentrations are given in Table 1. Manual mixing may be applied to mix two components.

Caution - **Use H_2O_2 at a concentration not exceeding your predetermined limits.**

Table 1. Examples of starting H_2O_2 Concentration Required at Various Substitution percentages and Gel Concentrations

Substitution (%)	1.5	1.5	1.5
Gel concentration (mg/mL)	10	25	40
H_2O_2 for cross-linking (%)	0.04	0.09	0.14

Substitution (%)	2.8	2.8	2.8
Gel concentration (mg/mL)	20	30	40
H_2O_2 for cross-linking (%)	0.13	0.20	0.27

Substitution (%)	5.5	5.5	5.5
Gel concentration (mg/mL)	30	40	50
H_2O_2 for cross-linking (%)	0.39	0.52	0.65

Storage

- Upon hydration, if not immediately used, TS-NaHy solution should be stored at: 2 – 8°C to minimize microbial growth. Prolonged storage is not recommended.
- If not immediately used, the crosslinked gel should be stored at 2 – 8°C to minimize microbial growth.

FOR RESEARCH USE ONLY - **NOT FOR HUMAN USE.**

Technology covered by U.S. PATENTS – 6,982,298; 7,368,502; 7,465,766 and related foreign patents.

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