



Thermacol® Kit

Product Information Sheet

09/12/18 Rev. 2.0

The Thermacol® Kit has all the components for creating 3D cell-seeded bioartificial type I collagen gels (see Table 1) with the addition of telopeptide-containing collagen, which provides the mix with greater stability at low temperature (4 °C), different physical properties upon gelation, and rapid and controllable gelation kinetics. For more information, see the Thermacol® product webpage at <http://www.flexcellint.com/Thermacol.htm>.

Table 1. Thermacol® Kit materials per kit size

Component	Chemical	Volume (ml)		
		Mini	Midi	Maxi
Collagel®	Atelopeptide-containing Type I Collagen (3 mg/ml in 0.01 M HCl)	8	16	32
Thermacol®	Telopeptide-containing Type I Collagen (3 mg/ml in 0.01 M HCl)	2	4	8
Reagent A	5X MEM	2	4	8
Reagent B	Fetal Bovine Serum	1	2	4
Reagent C	1 M HEPES	0.25	0.5	1
Reagent D	0.1 M NaOH in 5X MEM	0.5	1	2

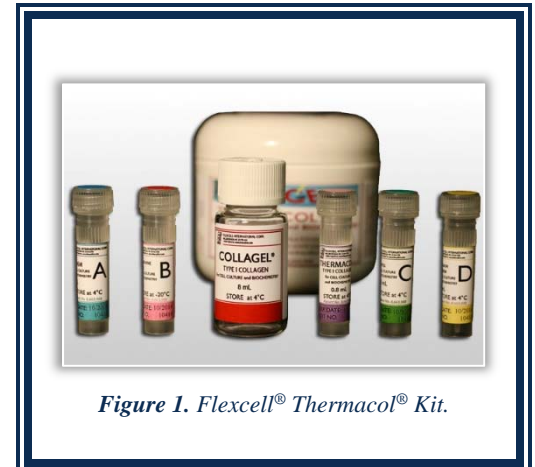


Figure 1. Flexcell® Thermacol® Kit.

PROTOCOL FOR PREPARATION OF 1 ML THERMACOL® COLLAGEN GEL FOR GENERAL USE

1. Reconstitute Reagent B (Fetal Bovine Serum, Lyophilized) in 1.2 mL of sterile water as instructed below.
2. In a 1.5 ml centrifuge tube, combine 160 µl reagent A, 80 µl reagent B, and 20 µl reagent C.
3. In a second 1.5 ml centrifuge tube, combine¹ 630 µl Collagel®, 70 µl Thermacol®, and 40 µl reagent D. After mixing well, ensure that the pH of the Collagel®/Reagent D mixture is 7.0 – 7.2. If not, add 2.5 µl of Reagent D, mix well, and test again. Repeat until pH is in desired range.
4. Combine the two tubes and mix well by pipetting.
5. This gel solution may be used to resuspend your cell pellet or other constituents for plating. See figure 2 for kinetics of gelation at 37°C.

¹ This is to produce a 10% Thermacol®/Collagel® gel. The ratio can be adjusted (0-20%) to produce gels that form at a different rate and have different physical properties. See figure 2.

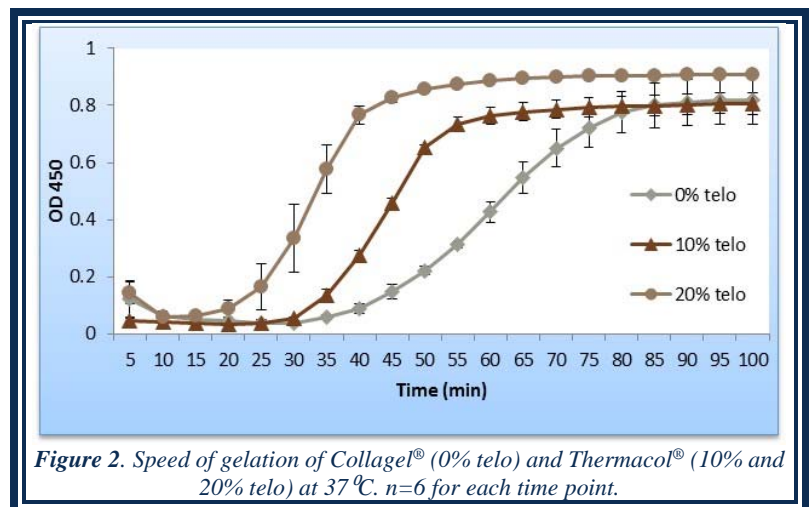


Figure 2. Speed of gelation of Collagel® (0% telo) and Thermacol® (10% and 20% telo) at 37°C. n=6 for each time point.

PROTOCOL FOR CASTING BIOARTIFICIAL CELL-POPULATED HYDROGELS (BAT) IN FLEXCELL® LINEAR TISSUE TRAIN® CULTURE PLATES

1. Prepare cells according to your established protocol for primary cultures or continuous cell lines in the medium of choice.
2. Release cells from their substrates with 0.05% trypsin, trypsin-EDTA, 0.05% bacterial collagenase, or other means.
3. Add serum containing media to the cells to neutralize the trypsin or collagenase.
4. Count cells and determine the number of cells needed, approximately, 50,000-200,000 cells in 150-200 µl Thermacol® collagen gel for each well of a 6-well Tissue Train® culture plate. NOTE: Cell seeding density in a 3D gel will vary depending on cell type. We recommend testing cell seeding densities to determine the best cell number for your application and cell type.
5. Wash cells 2x with medium to remove trypsin or collagenase.



6. Position Tissue Train® plate with gasket on top of a Trough Loader™ and ensure proper seating to achieve a sealed vacuum.
7. Initiate a maximum elongation (~90 KPa) static pull down regimen using the Flexcell® FX-5000™ Tension System. *Note: For standard Thermancol®, 2 hours of gelation time is recommended. It is also recommended that the vacuum be released slowly by 2% elongation of maximum every 6 seconds until 0% elongation is achieved.*
8. Preparing the Thermancol®-media matrix, see table below for example volumes. In a 1.5 ml tube (not supplied), combine reagents A, B and C. In a 5.0 ml tube (not supplied), combine Collagel® with Thermancol® (90% Collagel® to 10% Thermancol®) then add reagent D and mix well (thoroughly mixed solution should become light peach in color). Remove 10 µl of the Reagent D-Thermancol® mixture and apply to pH paper (not supplied) to ensure pH is neutral (7.0-7.2 is ideal). *Note: If not, add 2.5 µl Reagent D and check again. Repeat until pH is in desired range.* Add Reagent A, B, C mixture to the Thermancol® mixture and mix well by pipetting. *Keep all reagents at 4°C or prepare on ice.*

Reagent	Volume (µl) (Example Ratios)	6-Well Tissue Train® (200 µl/BAT)	24-Well HTP Tissue Train® (50 µl/BAT)
A	160	288 µl	288 µl
B	80	144 µl	144 µl
C	20	36 µl	36 µl
Collagel®	630	1134 µl	1134 µl
Thermancol®	70	126 µl	126 µl
D	40	72 µl	72 µl
Total	1000	1800 µl	1800 µl

9. Working quickly, resuspend the cell pellet in the complete Thermancol®-media matrix and mix well by pipetting up and down.
10. Micropipette 200 µl cell constituted Thermancol®-matrix into each linear trough of a Tissue Train® plate. First pipette a small drop of gel at each end of the trough, under the anchor stems. Then press the anchor stems into the trough and release several times, thoroughly wetting the tabs. Finally, fill the middle of the trough with gel, moving the pipette back and forth to create a uniform strip of gel in the well (see video of *Tissue Train® Bioartificial Tissue Fabrication with Uniaxial Strain* on Flexcell®'s web site, www.flexcellint.com).
11. Place baseplate into 37 °C 5% CO₂ incubator for 2 hours and allow the constructs to polymerize.
12. Near the end of the static pull down (approximately 10 minutes remaining) while the vacuum is still engaged, transfer baseplate to a tissue culture hood and add 3.0 ml complete growth media making sure to pipet very slowly to prevent undue motion to the gelling BATs.
13. Once vacuum has released, remove plates and place in incubator at appropriate growth conditions. Culture constructs according to the laboratories established protocol. It is recommended that the growth media be changed every 3 days.

PROTOCOL FOR PREPARATION OF FETAL BOVINE SERUM (REAGENT B)

1. Add 1.2 mL of sterile DI water to the lyophilized serum with a sterile 5 mL syringe and 22-gauge needle. Resuspend without foaming.
2. Draw the resuspended serum into the 1 mL syringe. Remove the 22-gauge needle and apply a 0.22 µM syringe filter to the 5 mL syringe. Express the reconstituted serum into a sterile 2 mL vial.
3. Store filtered solution at 4 °C. Use within one-two weeks of preparation. Alternatively, aliquot the reconstituted FBS in 100 µL volumes and store at -20 °C until ready to use.
NOTE: User can also use FBS from their own source in place of the lyophilized FBS kit component Reagent B (Test source FBS to be sure the serum works in the kit before large scale use).

RECOMMENDED REAGENT STORAGE CONDITIONS

All kit components can be stored at 4 °C.

ORDERING INFORMATION

Thermancol® Kits are sold in 3 sizes: Mini (Cat. No. ThermaKIT-100A), Midi (Cat. No. ThermaKIT-100B), and Maxi (Cat. No. ThermaKIT-100C). A Mini kit has approximately enough solution to make BATs in eight 6-well linear Tissue Train® culture plates, a Midi kit has enough for sixteen 6-well plates, and a Maxi kit for thirty-two 6-well plates.

Flexcell® Collagel® and Thermancol® are protected by the following patents: US Patents 8,663,988; 8,857,500; 8,993,325; 9,018,009.