Technical Data Sheet (TDS)

Product Name: BIO SIRA

Catalogue Number: BVBA100

BVBA50

BVBA20

1. Introduction:

Bio Sira – Atelocollagen Type I is an advanced biomaterial developed from bovine sources through a proprietary **extraction and purification process**.

Bio Sira is a sterile, **high purity (>98%)**, Atelocollagen Type I, supplied in a lyophilized form ensuring convenience and long-term stability. Bio Sira offers researchers a reliable substrate that closely mimics the extracellular matrix (ECM), enabling enhanced **cell adhesion and growth.**

2. Product Description:

- High Purity: ≥98% (confirmed by Silver Staining)
- Triple-Helical, Fibrillar Structure: Maintained for optimal performance
- Biocompatibility: Reduced immunogenicity with reliable cell attachment and viability
- Reproducibility: Consistent lot-to-lot performance
- Made in India product.
- Fast Delivery: Available within one week.

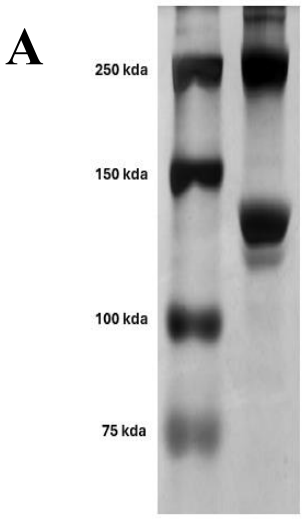

Figure A. SDS-PAGE Analysis of Type I Collagen

Silver-stained SDS-PAGE gel illustrating the purity of Type I collagen. **Lane 1**: Molecular weight marker. **Lane 2:** Type I collagen showing distinct bands corresponding to the α1 and α2 chains, along with the β component, confirming the structural integrity and purity of the protein.

3. Key Technical Features:

Category	**Details**
Source	Buffalo
Form	White, Lyophilized Powder
Purity	≥98% (Silver Staining)
Structure	Triple-Helical, Fibrillar
Sterilization	Filtration
Endotoxin Level	≤ 0.5 EU/mL (LAL Assay)
Packaging	10 mg, 50 mg, 100 mg vials
Appearance	White, puffy lyophilizate
Shelf Life	24 months (lyophilized, –20°C to –80°C)
Reconstituted Stability	Up to 6 months at 2–8°C

4. Key Technical Features:

Component	Description
Lyophilized Atelocollagen Type I	Available in 10 mg, 50 mg, or 100 mg vials (depending on pack size)
Product Information & Technical Data Sheet	Detailed specifications and usage guidelines
Reconstitution Guidelines	Step-by-step instructions for Bio Sira dissolution and preparation
Certificate of Analysis (CoA)	Provided with every batch
Safety Data Sheet (SDS)	Available upon request

5. Reconstitution Guidelines

- Dilute the lyophilized material to 5mg/mL using 0.02M CH3COOH.
  Note: Chill everything (tube, acid), work on ice, pH 2-3 prevents premature fibrillogenesis.
- Gentle rehydration: Initially, wet the powder walls with a few µL, then bring to final volume.
- Gently mix the solution by slow end-over-end rotation or rocking at 4^0C overnight (12-18 hours).
  Do not vortex; this will avoid bubbles and foam.
- Clarify by slow spin at 3000 g, 5 min, 4 °C until it dissolves completely.
- Post completely dissolved, store at 4°C and do not freeze.

Suggested Coating Procedure – (Refer to the attached sheet at the end of this TDS)

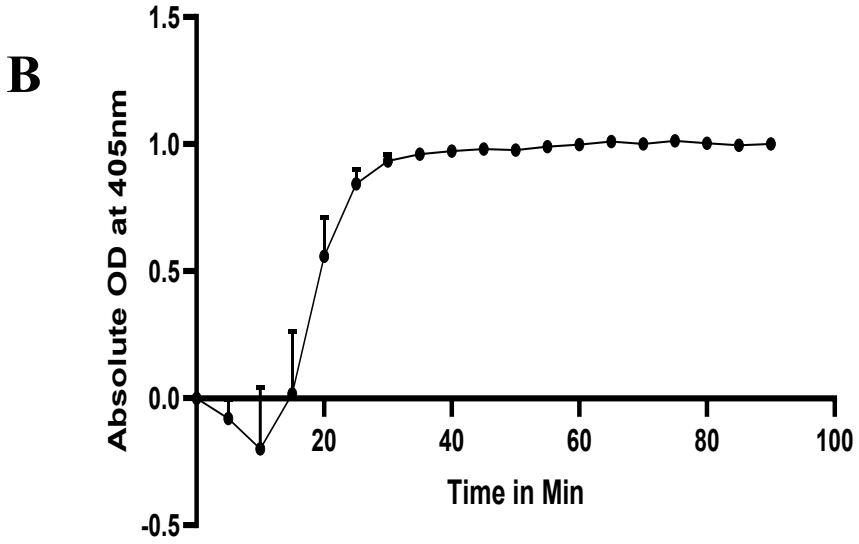

Figure B. Hydroxyproline Quantification in Type I Collagen

Quantitative analysis of hydroxyproline in Type I collagen samples. Increasing collagen concentrations show a proportional rise in hydroxyproline content, validating the integrity and compositional quality of the collagen preparation. Error bars represent mean ± SEM.

6. Applications

- 3D Cell Culture & Organoid Models
- Tissue Engineering & Scaffold Development
- Regenerative Medicine Studies
- Wound Healing Models
- 3D Bioprinting
- Injectable Hydrogels
- Drug Delivery Systems
- Stem Cell & Organoid Research

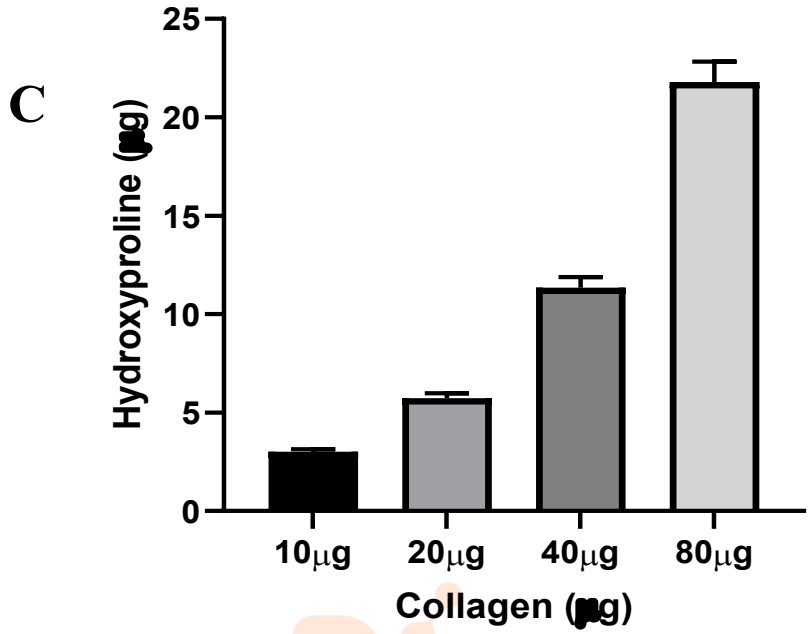

Figure C. Gelation kinetics of the sample.
Time-dependent increase in absorbance at 405 nm indicates gel formation. The sample shows a rapid rise in optical density within the first 30 minutes, reaching a plateau, suggesting that complete gel formation occurs within 40 min. Data are presented as mean ± SEM (n = 2).

7. Safety & Handling

- Classified as non-hazardous under standard laboratory conditions.
- Recommended PPE: gloves, lab coat, and eye protection.
- Handle under aseptic conditions to avoid contamination.

8. Storage & Stability

- Lyophilized form: Store at 2 °C to 8 °C; stable for 24 months.
- Reconstituted form: Store at 2–8 °C; stable up to 6 months.
- Protect from repeated freeze thaw cycles.

9. Ordering Information

Catalogue No.	Product	Pack Size
BVBA100	Bio Sira – Atelocollagen Type I	10 mg
BVBA50	Bio Sira – Atelocollagen Type I	50 mg
BVBA20	Bio Sira – Atelocollagen Type I	100 mg

10. Intended Use

For Research Use Only (RUO).

11. Contact Information

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For Research Use Only.

Type 1 Collagen coating for cell culture

Aim: To create a uniform collagen substrate that enhances cell adhesion, morphology and viability for invitro assays.

Materials Requirements:

- Lyophilized collagen (BIOSIRA)
- 0.02M Acetic acid
- Falcon tube
- Gel rocker
- pH strips
- 7.5% NaHCO3
- 0.01 N Hcl
- 1X PBS (Sterile-Cold)
- 96 well plate with lid

Dissolve lyophilized collagen:

- Dilute the lyophilized material 5mg/mL using 0.02M CH3COOH.
  Note: Chill everything (tube, Acid), work on ice, pH 2-3 prevents premature fibrillogenesis.
- Gentle rehydration: wet the powder walls initially with a few µL, then bring to final volume.
- Gently mix the solution by slow end-over-end rotation or rocking at 4^0C overnight (12-18 hours). Do not vortex: Avoid bubbles/foam.
- Clarify by slow spin at 3000g,5 min, 4^0C until it dissolves completely.
- Post complete dissolved, store at 4°C and do not freeze
- Estimate the total protein content using Biuret assay.

Neutralization:

- Collagen in 0.02M CH3COOH, 600µg (0.2mLof 3mg/mL)
- By adding drop wise (1-2 µL at a time) Adjust the collagen pH to 7.0-7.2 using 7.5% NaHCO3.
- Post reaching target pH 7.0-7.2, immediately bring close to 1 mL using 1X PBS.

Casting and Gelation:

Table:1

Gel Thickness	Volume for 0.32 cm^2 in µL	Notes
1.0 mm	30-40	Minimum stable layer
1.5 mm	50-75	Common choice (Good nutrient diffusion)
2.5 mm	80-100	Used if cells are highly contractile or dense.

- Add (30-40µL per well volume for thin coating and 80-100µL for thick coating) neutralised collagen solution as prepared above.
- Incubate at 37°C for 40 min-60 minutes to promote gelation.
- Post gelation, store the plate at 2-8°C, do not freeze.
- Minimize opens and avoid contamination and Typical shelf life is not more than one month.

Methods for Embedding and seeding cells in Collagen Hydrogels

Description:

This protocol provides step by step procedures for culturing mammalian cells using collagen hydrogels as a 3D Extracellular matrix. It describes two commonly used approaches for introducing cells:

1.Cell seeding on top of pre-formed collagen hydrogels-where cells are plated onto the gel surface, mimicking a 2D-3D interface.

2.Cell encapsulation within collagen hydrogels-where cells are suspended in neutralized collagen solution prior to gelation, resulting in uniform 3D embedding.

Materials requirements:

- BIOSIRA (Neutralized)
- Cell lines of interest.
- Appropriate complete culture medium
- Trypsin-EDTA solution (0.25%)
- Culture-multi well plates
- Tissue culture flasks
- Sterile centrifuge tubes
- Sterile serological pipettes
- Micropipettes with sterile tips
- Ice bucket with ice.

Procedure:

Culture the cells according to the manufacturer's protocol. Harvest and collect the cells by centrifugation to obtain a pellet and keep the cell pellet on ice to preserve cell viability.

Exp:1

Cell seeding on pre-formed Collagen Hydrogel

- Dispense the required volume of chilled neutralized collagen solution into each well (refer Table 1), ensuring the bottom surface is completely covered.
- Cover the plate with its lid and incubate at 37°C with 5% CO2 to allow gelation-at least 60 minutes.
- Resuspend the pellet in fresh culture medium at the desired seeding density
- Note: Vary depending on the cell type, growth characteristics and experimental requirements (e.g., 100,000 cells in 0.1mL for a 96 well plate). Adjust both cell number and medium volume proportionally for different cell lines and plate formats.

- Carefully add the cell suspension on top of the solidified collagen hydrogel.
- Incubate the plate at 37^0C with 5% CO2 under standard culture conditions.

Exp:2

Encapsulation of cells within collagen hydrogels

- From your cell pellet tube, carefully aspirate the spent culture medium from the cell pellet without disturbing the cells. Immediately resuspend the pellet in pre-chilled neutralised collagen solution.
- Mix the cell suspension gently by pipetting up and down with a pre-chilled serological pipette to ensure homogenous distribution of cells within the collagen solution. Avoid vigorous pipetting to minimize bubble formation.
- **Note:** For Higher conc (e.g.,2-3 mg/mL): Cells may be resuspended in a small volume of culture medium before mixing with the collagen solution, this step helps disperse the pellet and prevent clumping, while the minor dilution will not significantly compromise gel integrity.
- For lower conc (e.g., <1mg/mL) To avoid weakening the gel, the pellet should be added directly to the neutralized collagen solution. If the pellet is dense and prone to clumping, it may first be gently resuspended in a minimal volume of medium to achieve a uniform suspension, and then immediately mix with the collagen
- Add collagen hydrogel precursor to cell culture plates. Recommended seeding volume per well is given in the above Table 1.

- Cover the culture plate with its lid and transfer it to a humidified incubator maintained at 37°C with 5% CO2. allow the collagen solution to polymerise undisturbed for at least 60 minutes to ensure complete gelation.
- Post gelation, gently add pre warmed cell culture medium to the well, ensuring the collagen gel is fully covered. Return the plate to the incubator and maintain the cultures under standard conditions (37°C, 5% CO2) for the desired culture period.