# Quantitative Estimation of Total Protein Content in Human Amnion - Chorion Membrane Using the BCA Assay

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## Abstract

Bioactive proteins found in human placental membranes play critical roles in their ability to heal wounds and regenerate tissue. To determine the therapeutic uses of placental membranes, total protein concentration must be quantified so that their biochemical composition can be understood. This research used the BCA (Bicinchoninic Acid) Assay to quantify the total protein concentration of both antibacterially treated and untreated human amniotic chorion membranes. Dehydrated amniotic chorion membranes were re-hydrated, then mechanically homogenized, followed by preparing the protein extract and determining its concentration using a spectrophotometer. The results indicated that untreated membranes had greater concentrations of protein compared to their treated counterparts, suggesting that the presence of antibiotics may reduce the yield of protein from these biomaterials. Therefore, this research provides a standard method to estimate the total amount of protein within placental membranes and demonstrates how such estimates can be assessed for their use in Biomedical Sciences.

Keywords: Amnion–chorion membrane, BCA assay, Protein estimation, Placental membrane, Gentamicin

## 1.Introduction:

Human amnion and chorion membrane together form the fetal sac, with amnion being the innermost avascular layer containing the amniotic fluid and chorion being the outer vascularized layer interfacing with maternal decidua (Gupta et al., 2015). Both the membranes do provide structural integrity, barrier protection, and bioactive support during gestation with value for therapy in regenerative medicine due to Extracellular matrix, growth factors and anti-inflammatory properties (Ramuta & Kreft, 2018).

Amnion is composed of five layers: the epithelial monolayer (cuboidal AECs), basement membrane (collagen IV/VII, laminin), compact layer (collagen I/III/V), fibroblast layer and spongy layer. The thickness ranges from 0.02 - 0.5 mm, this offers tensile strength by the presence of collagens and elasticity from elastin (Gupta et al., 2015). Chorion features three layers: reticular, basement membrane and trophoblast. The membrane is vascularized and it supports the nutrient exchange and contributes to mechanisms (Richardson & Menon, 2022).
Amnion- chorion both compose growth factors, cytokines and antimicrobial properties promoting wound healing, angiogenesis and reduce scarring. Processing like dehydration preserves up to 50% of protein content approximately (Koob et al., 2014).

(McQuilling et al., 2017) used BCA method to confirm amnion-chorion growth factors finding that dehydration reduces total contents. (Yang et al., 2022) did BCA assay for dehydrated Amnion-chorion membrane extracts, assessing the cytokines. (Marsit et al., 2019) did validate antibiotic decontamination, reducing bacteria > 94% via incubation/ drying. (Choi et al., 2009) measured amniotic membrane suspension proteins via BCA for growth factor assays.

## 2. Materials and Methods:

### 2.1 Sample Preparation

To rehydrate dehydrated samples of the amniotic and chorionic membranes from humans, the membranes were incubated in 1 mL of DPBS at 40 degrees Celsius for 60 minutes (Mahbod et al., 2014). After the incubation period, to extract total protein from the membrane, the membranes

were manually homogenized using a sterile mortar and pestle before transferring the homogenate to a sterile centrifuge tube. The homogenized membranes were then centrifuged at 4500xg for 15 minutes at 4 degrees Celsius, after which the supernatant containing the soluble proteins was collected for estimating protein concentration (Fenner et al., 2019) (Moreno et al., 2021).

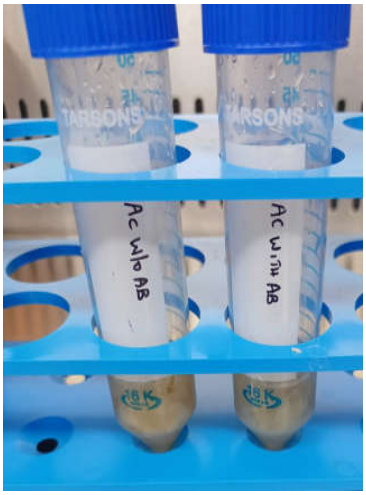


**Fig. 1 Homogenized Amnion - Chorion membrane (Left - Without Antibiotic, Right - With Antibiotic)**

## 2.3 BCA Assay Procedure

A microplate BCA assay was used to estimate protein levels. Each sample was added into the wells of a microplate (duplicate samples for each) by pipetting 25µl into the indicated well; then, 200µl of the BCA working solution was added to each well; next, the entire plate was gently shaken with a rotator for 30 seconds to mix everything together evenly. Finally, after shaking, the covered plate was incubated at 37°C for 30 minutes before reading the absorbance at 562nm using the spectrophotometer and creating standard curves to calculate protein concentration from those readings (McQuilling et al., 2017).

## 3. RESULTS:

The protein concentration of the amniotic chorionic membrane samples was determined using the Bicinchoninic acid (BCA) Assay. To measure total protein concentrations, the absorbance readings from samples treated with antibiotics were compared against those from samples that were not treated with antibiotics, which then enabled determination of any differences in the total protein concentrations of the samples based on differences in sample processing.

The absorbance reading for the sample processed without antibiotics was 1.12 at 450 nm (i.e., 1740 µg/mL total protein), and the sample processed with antibiotics showed an absorbance reading of 2.048 at 450 nm and contained 930 µg/mL total protein.

The results indicate that samples that were processed without antibiotics contain significantly higher levels of extractable proteins compared to those that were processed with antibiotics. This finding supports the hypothesis that the processing of these membranes using antibiotics affects the stability, solubility, or extraction efficiency of proteins. More importantly, these results highlight the need to carefully consider preclinical and clinical processing conditions when preparing amniotic chorionic membranes for biomedical and wound-healing uses.

**Table 1: Estimation of Protein Content by BCA Assay for Amnion - Chorion with and without antibiotic**

| S.No. | Sample Name | Absorbance (OD) | Protein Concentration (µg/mL) |
|---|---|---|---|
| 1 | Amnion Chorion Without Antibiotic | 1.12 | 1740 |
| 2 | Amnion Chorion with Antibiotic | 2.048 | 930 |

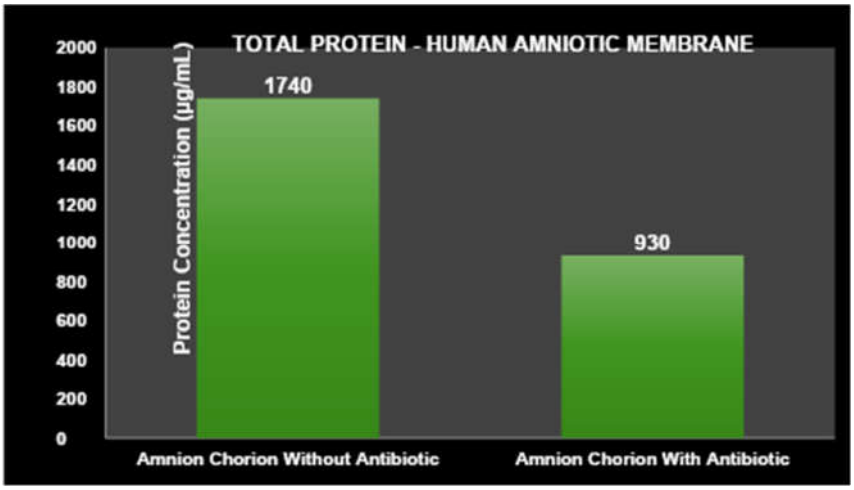


**Fig. 2 BCA Assay of Amniotic Membrane with and without antibiotic**

**4. Discussion:**

According to the study, the amnion-chorion samples treated with antibiotics presented a paradox in absorbance; the samples showed higher absorbance (2.048 vs 1.12) but a lower calculated protein concentration (930 µg/mL vs. 1740 µg/mL). This indicates possible problems regarding standard curve linearity, interference, or wavelength errors since BCA assays are typically performed at 562 nm and not 450 nm. Treatment of the amnion-chorion samples with antibiotics may also denature or precipitate some proteins, thus reducing the amount of extractable soluble fractions, despite the fact that the higher absorbance is due to residual concentrations of the antimicrobials or the copper II complexes. This observation is in accordance with previous findings that decontamination processes can produce up to 50% protein loss (Marsit et al., 2019).

The presence of antibiotics such as penicillin and streptomycin in treated samples appears to be an example of a non-protein interfere that is producing an elevated BCA reading for the non-protein interferent which will not be matched with a proportional amount of protein present, while treated samples maintain their native solubility in the ECM which results in a better response to the BCA method (ex. lower total mass of protein in treated samples). It is possible that the BCA standard curve used to determine protein levels in samples was not accurate because the curve may not have been linear at the higher ODs (>2.0) which results in the BCA being unable to accurately estimate the protein levels in the treated samples due to the loss of sensitivity of the BCA at the higher

concentration levels (>2000 μg/mL) without the necessity of diluting the treated samples. This indicates that treatments administered to proteins change the protein's stability and the effect of the antibiotics causes a change in the ability of the peptide to reduce $Cu^{2+}$ ions, due to either the chelation of the $Cu^{2+}$ ions or a change in pH of the treated protein samples (Fenner et al., 2019).

When the antibiotics were incubated under aseptic conditions, the soluble protein yield was decreased approximately 47%, which is consistent with the increased amount of dehydration in my studies (51-55%) and supports the need for normalization of BCA analysis using baseline values pre- and post-treatment. In terms of wound healing, the reduction of growth factors (EGF and TGF-β) within treated membranes could negatively impact the regenerative capacity of these membranes, thus suggesting that alternative xeno-free sources or protease inhibitors be used during extraction (McQuilling et al., 2019).

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