

Comparison of two Casein grades as blocking agents in a hCG lateral flow assay

Introduction

Casein, an abundant milk protein, is used in several nucleic acid and immunological detection methods to reduce non-specific binding. Multiple grades of casein are commercially available that offer various levels of purity as well as solubility. Bovine Casein, C7078, supplied by Sigma-Aldrich is a technical grade casein that contains multiple casein subunits as well as protein impurities and a higher fat content than partially purified caseins. Recommended preparation instructions for C7078 casein advise suspending the casein powder directly in 1 M NaOH (50 mg/ml). Other dissolution methods include increased temperature, pH, and shearing using ultrasonic and physical methods. This requires increased buffer preparation time and can cause inconsistency in the final buffer preparation. CaseiNSBlock, SRE0081, supplied by Sigma-Aldrich, is an alkali-treated casein formulated to improve solubility compared to other forms of casein, while also reducing non-specific binding and background signal.

A lateral flow assay (LFA) to detect the pregnancy hormone, human chorionic gonadotropin (hCG) was selected as a model to illustrate the performances of these two grades of casein (when used as a blocking buffer component during the conjugation)¹. Two different detector particles were utilized in this comparison: Estapor® Red Intense microspheres (K1-030 Red Intense, 0.3 microns), and gold nanoparticles (40 nm).

Estapor® Red Intense microspheres were conjugated to the hCG detection antibody (MABX2013) using a two-step EDC/Sulfo-NHS covalent coupling process² and gold nanoparticles were passively conjugated to antibody (using established methods). Performance of the conjugated detector particles in LFAs were assessed by assay sensitivity, assay curve shape, and background. Peak intensities (arbitrary units (a.u.)) of the Control and Test Lines were determined by the Axxin reader (AX-2X-S).

Differences in Casein Dissolution

C7078 casein takes between 2-6 hours to dissolve into solution. The variation in time to dissolution can be dependent on the lot of casein used, the buffer composition and/or the application of heat. In contrast, SRE0081 casein takes approximately 10-15 minutes to complete dissolution regardless of base buffer and does not require heat or pH adjustment.

Impact of casein grade used in buffer preparation on passively conjugated particles

Gold hCG conjugates demonstrate equivalency regardless of which grade of casein is used to prepare the conjugate blocking buffer. Background peak intensity levels at the test line (0 mIU/mL hCG) were 613 a.u. for conjugates blocked with C7078 and 556 a.u. for conjugates blocked with SRE0081 casein. Assay sensitivity and curve shape were also equivalent for hCG test strips regardless of which grade of casein was used in the conjugate preparation (**Figure 1 A and B**).

Fig 1A: SRE0081

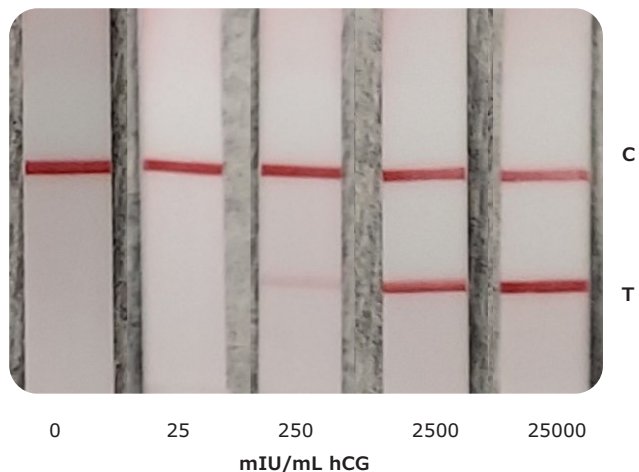
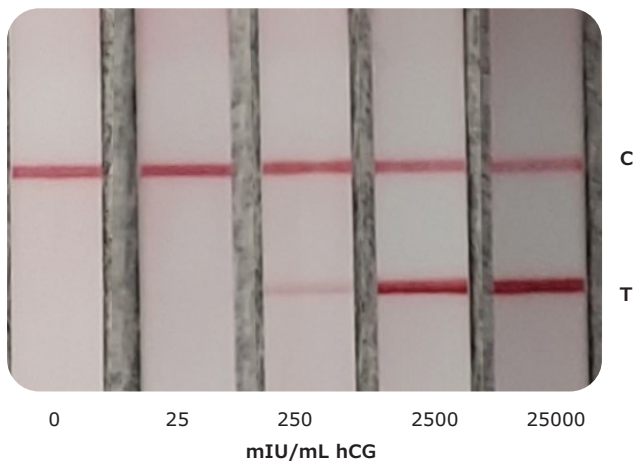


Fig 1B: C7078



Impact of casein grade used in the blocking buffer preparation for covalently conjugated particles

Anti-hCG antibodies were covalently conjugated to Estapor® Red Intense microspheres and blocked with either SRE0081 or C7078. Estapor® Red Intense microsphere conjugates were tested at two final conjugate concentrations, 0.005% and 0.01%. Conjugates blocked with SRE0081 at both 0.005% **Figure 2A** and 0.01% (data not shown) demonstrated intense sharp red lines and a sensitivity of 25 mIU/mL hCG. However, conjugates blocked with C7078 demonstrated a sensitivity of 250 mIU/mL hCG at 0.005% (**Figure 2B**). Increasing conjugate amount 2-fold to 0.01% was required to achieve the desired sensitivity of 25 mIU/mL hCG for conjugates blocked with C7078 (data not shown).

Fig 2A: SRE0081

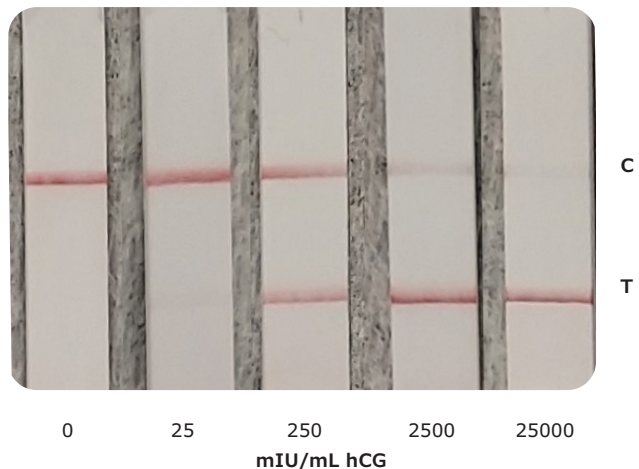
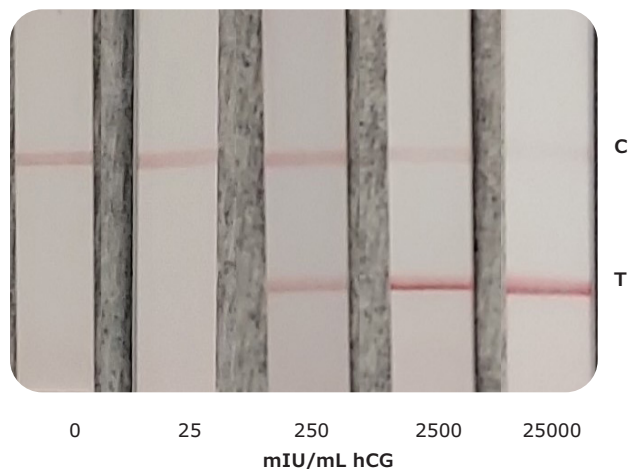


Fig 2B: C7078



Summary/Conclusions

The impact of using two different casein grades (C7078 and SRE0081) in conjugate buffer preparations for a hCG lateral flow assay were assessed. C7078 casein, the less costly option, requires more extensive buffer preparation time involving application of heat or pH adjustments to dissolve it. SRE0081 casein offers a quick dissolution time without any buffer manipulations thereby significantly reducing the required buffer preparation time.

Two different detector particle types (Gold and Estapor® Red Intense) were used in this comparative analysis. hCG test strips prepared with antibody conjugates that were passively conjugated to gold nanoparticles demonstrated equivalence regardless of whether SRE0081 or C7078 casein was used in the conjugation buffer. Antibody conjugates prepared with buffer using SRE0081 casein appeared to show enhanced sensitivity in the hCG assay compared to C7078 casein when covalently conjugated to Estapor® Red Intense microspheres. The impact of the Casein type used in conjugate buffer preparations for lateral flow assays should be assessed independently. In this model, SRE0081 casein offers enhanced consistency and reduced buffer preparation time therefore increasing the overall efficiency of the workflows and processing steps.

Table 1: Recommended buffers and solutions

Activation/Coupling buffer (Covalent Conjugation)	50 mM MES, pH 6.0
Blocking Buffer (Covalent Conjugation)	50 mM Tris, pH 8.0, 0.5% (w/v) casein
Buffer A (Passive Conjugation)	100 mM Potassium Phosphate pH 7.4
Buffer B (Passive Conjugation)	10 mM Potassium phosphate, 10% BSA pH 7.4
Buffer C (Passive Conjugation)	0.5X PBS, 0.5%BSA, 0.5% Casein, 1% Tween, pH 8.0
Conjugate Pad Buffer	50 mM Tris, pH 8.0, 0.5% (w/v) casein, 10% Sucrose, 2.5% Trehalose, 0.5% PVP pH 8.0
Sample Pad Buffer	10 mM Tris, 1% Tween 20, 0.75% BSA pH 8.2

References:

1. Application Note: 'Performance of Estapor® Microspheres and Hi-Flow™ Plus Membranes in a Lateral Flow Assay for Human Chorionic Gonadotropin (hCG)'. [MerckMillipore.com/estapor](https://www.merckmillipore.com/estapor)
2. Application Note: 'Microsphere Coupling, Two-step EDC/Sulfo NHS Covalent Coupling Procedure for Estapor® Carboxyl-modified Dyed Microspheres'. [MerckMillipore.com/estapor](https://www.merckmillipore.com/estapor)

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