

COLLAGEN CONJUGATION PROTOCOL

(most done in tissue culture hood)

Make 0.1N acetic acid in $\text{H}_2\text{O}^{\text{dd}}$ in the fume hood \rightarrow 285.7 μl for 50ml tube (store @ 4°C)

Make **1mg/ml stock in 0.1N ac.ac.** in tissue culture hood \rightarrow mix @ T_{room} for 1-3 hrs (store @ 4°C)

Make 50mM sodium acetate buffer, pH 4.5; filter \rightarrow 0.205g in $\text{H}_2\text{O}^{\text{dd}}$ for 50ml tube (store @ T_{room})

Calculate needed final collagen solution volume, $V = (\text{N gels}) \times (2\text{ml})$

Dilute stock to **0.05mg/ml in s.a. buffer** in tissue culture hood \rightarrow $(V \times 0.05)\text{ml}$ stock for $V\text{ml}$

Add **3.6 mg/ml NaIO_4 crystals** \rightarrow $(3.6 \times V)\text{mg}$ for $V\text{ml}$; twirl few times gently, don't shake

Let it sit 30min @ T_{room} in tissue culture hood to oxidize collagen

In tissue culture hood add 2ml of oxidized collagen solution per dish

Incubate for 1hr @ T_{room} in tissue culture hood (make sure UV is off)

Wash $3 \times 10\text{min}$ in PBS (replace PBS under the hood, put on top kim-wipe on outside shaker)

Store collagen-plated gels in PBS @ 4°C

NOTE: If plating cells immediately, add cell media instead of PBS and equilibrate ~1 hour in incubator before plating cells.

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