

Chromatin Assembly Protocol (S150 extract)

Components

10X McNAP (use 10 μ l per 100 μ l assembly reaction)

water	1	Order of addition IS important!
300 mM ATP	1	
0.1 M DTT	1	
0.1 M MgCl ₂	3	
1 M creatine phosphate (CP)	3	
ExB-5/50	0.9	
1 μ g/ μ l creatine phosphokinase	<u>0.1</u> for 10 μ l	

Assembly reaction:

Order of addition IS important, mix gently after addition of each component

Extract (S-150)	70 μ l (determine empirically for each extract)
ExB-5/50	16.4 μ l (adjust depending on volume of DNA and extract)
McNAP	10 μ l

Mix

Add DNA to side of tube

pHspXX3.2 (0.1 μ g/ μ l)	1 μ l (6.6 kb fragment; -1.5 to +1.8)
\emptyset X DNA (0.25 μ g/ μ l)	2.6 μ l (8 kb double strand)

Spin briefly to mix

Mix gently after spin

Incubate for 6 hours at 26° C (fly room)

Add 0.05% Sarkosyl to inactive endogenous chromatin remodeling complexes

Purify chromatin over Sepharose CL-4B spin column

Collect purified fraction into SILICONIZED tube and add BSA to 0.5 mg/ml

Stock solutions

300 mM ATP, pH 7.0	10 μ l aliquots at -80° C
1 M creatine phosphate (CP)	30 μ l aliquots at -80° C, (327.2 g/mol)
1 μ g/ μ l creatine phosphokinase (CPK)	prepare in ExB-5/50, 0.1% BSA; 10 μ l aliquots at -80° C do not freeze and thaw CPK, discard after use
0.1 M DTT	store at -20° C
0.1 M MgCl ₂	store at room temp

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Buffer ExB-5/50

10 mM HEPES, pH 7.6
0.5 mM EGTA, pH 7.9
5 mM MgCl₂
50 mM KCl
10% glycerol