

密閉式超音波破碎装置

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使用文献 & 参考資料リスト

付録：UCD-200型（出力200W）による処理例



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p.98「クロマチン免疫沈降法 (ChIP)」

執筆：京都大学大学院医学研究科先端領域融合医学研究機構 縣 保年 先生
京都大学ウイルス生体応答学研究部門生体防御研究分野 生田 宏一 先生

羊土社 実験医学別冊 「改訂第4版 新 遺伝子工学ハンドブック」
p.166「ウイルスによる遺伝子導入法」

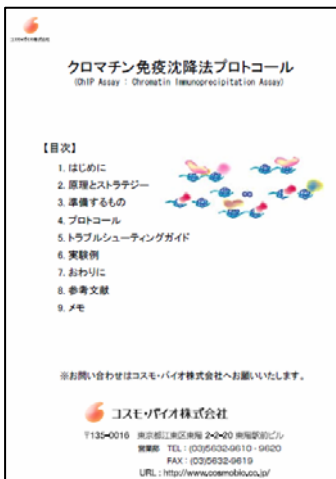
執筆：東京大学医科学研究所遺伝子解析施設 鐘ヶ江裕美先生、斎藤 泉先生

秀潤社 細胞工学 Vol.20 No.5 2001 5月号

p.752「RNase-richな好酸球からのRNA抽出」

執筆：国立療養所三重病院研究室 加藤佳子先生、藤澤隆夫先生

コスモバイオ 「クロマチン免疫沈降法プロトコール」



監修

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コスモバイオホームページ

各社技術情報 -クロマチンと遺伝子etc. (アブカム社 アップステート社 サンタクルズ社)



密閉式超音波細胞破碎装置
UCD-200型（出力200W）
による処理例

サンプル	容器	サンプル量及び濃度	処理時間	破碎・溶解状況
1. マウスの心臓(1mm角に切ったもの)	① 10mlスピッツ	① 0.5ml	① 10sec破碎×10sec休止×45回(15min)	① ほぼ100%破碎
2. 乳酸菌 (Lactobacillus, Lactobacillus acidophilus group菌)	① 10mlスピッツ	① 0.7ml 濃度 10e10~10e11/ml	① 20sec破碎×20sec休止15回 (10min)	
3. 培養細胞 (Chinese hamster lung fibroblast)	① 1.5mlチューブ	① 0.2~0.3ml 細胞浮遊液 (in PBS pH7.4)	① 10sec破碎×20sec休止×25回 (12.5min)	
4. 脂溶性酵素基質のミセル化	① 1.5mlチューブ	① 0.2~0.3ml	① 10sec破碎×20sec休止×25回 (12.5min)	
5. アデノウィルス培養細胞 (293細胞)	① 50ml スピッツ(ファルコン社 コーニング社住友社製等)	① 15ml	① 30sec破碎×30sec休止×4回 (4 min)	
6. MRSA (Staphylococcus aureus) の破碎	① 10mlスピッツ	① 200ul 濃度一昼夜培養後その 1/200容量をとり37℃で O.D600=2.7まで培養し集 菌洗浄後 50mM/HCL(pH7.5)5mMED TAで1/5にサスペンドしたもの	① 30sec破碎×30sec休止×40回(40 min)	① 生菌率1% Nite:50ul(1.5ml チューブ)では上記 条件で生菌率は 60%。
7. 大腸菌	1) ① 50ml スピッツ (標準タイプ) ② 50mlファルコン チューブ	① 20ml ② 15ml サンプル濃度 上記両ケース共 500mlでO.D1.1-1.2で集菌 シバファーに調合し25-30mlの サンプルを得る。	① 20sec破碎×20sec休止×6回(4min) ② 20sec破碎×20sec休止×12回(8min)	両条件でほぼ同 程度(約90%)の 破碎結果が得ら れた。
8. 大腸菌	2) ① 10mlス ピッツ ② 10mlスピッツ	① 1ml ② 1ml ①濃度 2-3×10e9個/ml ②濃度 10e7 - 10e8個/ml	① 30sec破碎×30sec休止×4回(4min) ① 30sec破碎×30sec休止×8回(8min) ② 30sec破碎×30sec休止×4回(4min)	① 95%以上破碎 ① 100%破碎 ② 100%破碎
9. 大腸菌 (DH5α/p T7Blue-xysB cells)	1) ① 10ml スピッツ ② 10mlスピッツ ③ 10mlスピッツ	① 0.5ml ② 1ml ③ 2ml ①②③濃度は同一3×10e9 個/ml	① 30sec破碎×30sec休止×5回(5min)、30sec破碎×30sec休止×10回 (10min) ② 30sec破碎×30sec休止×5回(5min)、30sec破碎×30sec休止×10回 (10min) ③ 30sec破碎×30sec休止×5回(5min)、30sec破碎×30sec休止×10回 (10min)	① 95%以上破 碎、100%破碎 ② 約75%破碎、 約90%破碎 ③ 約50%破碎、 約75%破碎

10.大腸菌 (DH5α/p T7Blue-xysB cells)	2) ① 1.5ml チューブ [®] (TPX製) ② 1.5ml チューブ [®] (TPX製)	① 300ul ② 250ul ①濃度 3×10 ⁹ 個/ml ②濃度 3×10 ⁹ 個/ml	① 30sec破砕×30sec休止×5回(5min)、30sec破砕×30sec休止×10回(10min) ② 30sec破砕×30sec休止×5回(5min、30sec破砕×30sec休止×10回(10min))	① 90%以上破砕、95%以上破砕 ② 約60%破砕、約85%破砕
11.クロレラ (Chlorella kessleri 211-11h (wild type, green) , C.kessleri 9.8 (white mutant)	1)① 1.5ml チューブ [®]	① 100ul ①濃度 100ulPCV/ml	① 30sec破砕×30sec休止×30回(30min)	① 95%以上破砕
12.クロレラ (Chlorella kessleri 211-11h (wild type, green) , C.kessleri 9.8 (white mutant)	2) ① 10ml スピッツ ② 10ml スピッツ ③ 10ml スピッツ	① 0.5ml ② 1ml ③ 2ml ①②③濃度は同一 100ulPCV/ml	① 30sec破砕×30sec休止×1回(1min)、30sec破砕×30sec休止×3回(3min) ② 30sec破砕×30sec休止×1回(1min)、30sec破砕×30sec休止×3回(3min) ③ 30sec破砕×30sec休止×1回(1min)、30sec破砕×30sec休止×3回(3min)	① 80%破砕、95%破砕 ② 60%破砕、92%破砕 ③ 30%破砕、70%破砕
13. クロレラ (Chlorella kessleri 211-11h (wild type, green) , C.kessleri 9.8 (white mutant)	3) ① 50ml スピッツ ② 50ml スピッツ	① 10ml ② 20ml ①②濃度は同一 100ulPCV/ml	① 30sec破砕×30sec休止×10回(10min) ② 30sec破砕×30sec休止×10回(10min)	① 90%破砕 ② 90%破砕
14.DNA (マウス genomic DNA) の切断	1) ① 1.5ml チューブ [®] (6本懸け)	① 50ul 濃度 不明	① 30sec破砕×30sec休止×3回(切断サイズにより調整) (3min)	① 1kbpを中心に10kbpから100bpの範囲でブロードに切断
15.DNA (マウス genomic DNA) の切断	2) ① 0.5ml チューブ [®] (12本懸け)	① 20ul 濃度 不明	① 30sec破砕×30sec休止×3回(切断サイズにより調整) (3min)	① 1kbpを中心に10kbpから100bpの範囲でブロードに切断
16.RNAの抽出 (RNase-richな抗酸球から) 細胞工学 Vol20 No5 2001p752参照	① 10ml スピッツ	① 1ml 濃度 1×10 ⁶ 個 (抗酸球は D16negative selectionで100%純度に分離後サイトカイン添加培地で6時間cultureし軽く遠心してペレットにしTRI Reagent 1mlを加えてすばやくVortexのHi スピート [®] で15秒間かけ全体を均一化しBioruptorにかける。)	① @130W 15sec破砕×15sec休止×2回(1min)	
17.試薬の溶解 (コルチゾール、テキサメサゾン等難水溶性の試薬)。ルチゾールの水への溶解	① 15ml チューブ [®]	① 12.5mlにHydrocortisolを0.4mg加えて溶解。(10e-4mol)	① @200W 6sec破砕×6sec休止×12回(2.4min)	① 溶解し変性もなかった。