密閉式超音波破砕装置

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付録：UCD-200型（出力200W）による処理例

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「クロマチン免疫沈降法プロトコール」

監修

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密閉式超音波細胞破砕装置 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	破砕・溶解状況
1. マウスの心臓(1mm角に切ったもの)	① 10mlｽﾋﾟｯﾂ	① 0.5ml	① 10sec破砕×10sec休止×45回(15min)	① ほぼ100%破砕
2.乳酸菌（Lactobacillus, Lactobacillus acidophilus group菌）	① 10mlｽﾋﾟｯﾂ	① 0.7ml 濃度 10^{10}～10^{11} 個/ml	① 20sec破砕×20sec休止15回（10min）	
3.培養細胞（Chinese hamster lung fibroblast）	① 1.5mlﾁｭｰﾌﾞ	① 0.2～0.3ml 細胞浮遊液(in PBS pH7.4)	① 10sec破砕×20sec休止×25回 (12..5min)	
4.脂溶性酵素基質のミセル化	① 1.5mlﾁｭｰﾌﾞ	① 0.2～0.3ml	① 10sec破砕×20sec休止×25回 (12..5min)	
5.アデノウィルス培養細胞（293細胞）	① 50ml ｽﾋﾟｯﾂ(ﾌｧﾙｺﾝ社ｺｰﾆﾝｸﾞ社住友社製等)	① 15ml	① 30sec破砕×30sec休止×4回（4 min ）	
6.MRSA (Staphylococcus aureus) の破砕	① 10mlｽﾋﾟｯﾂ	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)5mMEDTAで1/5にｻｽﾍﾟﾝﾄﾞしたもの	① 30sec破砕×30sec休止×40回(40 min)	① 生菌率1% Nite:50ul(1.5mlﾁｭｰﾌﾞ)では上記条件で生菌率は60%。
7.大腸菌	1） ① 50mlｽﾋﾟｯﾂ（標準ﾀｲﾌﾟ） ② 50mlﾌｧﾙｺﾝﾁｭｰﾌ	① 20ml ② 15ml ｻﾝﾌﾟﾙ濃度 上記両ｹｰｽ共500mlでO.D1.1-1.2で集菌しﾊﾞﾌｧｰに調合し25-30mlのｻﾝﾌﾟﾙを得る.	① 20sec破砕×20sec休止×6回(4min) ② 20sec破砕×20sec休止×12回(8min)	両条件でほぼ同程度(約90%)の破砕結果が得られた。
8.大腸菌	2) ① 10mlｽﾋﾟｯﾂ ② 10mlｽﾋﾟｯﾂ	① 1ml ② 1m l ①濃度 2-3×10^9個/ml ②濃度 10^7－10^8個/ml	① 30sec破砕×30sec休止×4回(4min) ① 30sec破砕×30sec休止×8回(8min) ② 30sec破砕×30sec休止×4回(4min)	① 95%以上破砕 ① 100%破砕 ② 100%破砕
9.大腸菌 (DH5α／pT7Blue-xysB cells)	1） ① 10mlｽﾋﾟｯﾂ ② 10mlｽﾋﾟｯﾂ ③ 10mlｽﾋﾟｯﾂ	① 0.5ml ② 1ml ③ 2ml ①②③濃度は同一3×10^9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) ③ 30sec破砕×30sec休止×5回(5mini)、30sec破砕×30sec休止×10回(10min)	① 95%以上破砕、100%破砕 ② 約75%破砕、約90%破砕 ③ 約50%破砕、約75%破砕

10.大腸菌 (DH5α／ｐT7Blue-xysB cells)	2) ① 1.5ml ﾁｭｰﾌﾞ(TPX製) ② 1.5mlﾁｭｰﾌﾞ(TPX製)	①300ul ②250ul 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ﾁｭｰﾌﾞ	①100ul 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ｽﾋﾟｯﾂ ② 10mlｽﾋﾟｯﾂ ③ 10mlｽﾋﾟｯﾂ	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ｽﾋﾟｯﾂ ② 50mlｽﾋﾟｯﾂ	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 ﾁｭｰﾌﾞ(6本懸け)	① 50ul 濃度 不明	① 30sec破砕×30sec休止×3回(切断ｻｲｽﾞにより調整) (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 ﾁｭｰﾌﾞ(12本懸け)	① 20ul 濃度 不明	① 30sec破砕×30sec休止×3回（切断ｻｲｽﾞにより調整）(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 ｽﾋﾟｯﾂ	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し軽く遠心してﾍﾟﾚｯﾄにしTRI Reagent 1mlを加えて すばやくVortexのHi ｽﾋﾟｰﾄﾞで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(ｺﾙﾁｿﾞｰﾙﾃﾞｷｻﾒｻｿﾞﾝ等難水溶性の試薬)。 ルチゾールの水への溶解	① 15mlﾁｭｰﾌﾞ	① 12.5mlにHydrocortisolを0.4mg加えて溶解。（10^4mol）	① @200W 6sec破砕×6sec休止×12回(2.4min)	① 溶解し変性もなかった。