BIOTECH SUPPORT GROUP

ProCipitate™

Superior Substitute to Phenol/Chloroform for DNA & RNA Isolation

- Removes only the contaminants & leaves DNA alone
- Improves yield of DNA over alternative bind and elute systems
- Adaptable to any sample size, and can be automated
- Key component of the ProPrep™ line of application specific kits

ProCipitate™ is a unique protein extraction reagent based upon patented elastomeric polyelectrolytes. The polymer chains of ProCipitate™ are initially extended in a high energy state due to an overall net negative charge. When introduced to protein solutions, the charges are neutralized and the polymer chains collapse to a more favorable energy state; DNA and RNA remain unreacted.

ProCipitate™ is non-hazardous and can replace phenol/chloroform with the additional benefits of solid-phase suspensions: adaptability to filtration and automation. It is routinely used for Plasmids, Cosmids, BACs, and Genomic DNA, as well as RNA. ProCipitate™ can also be used to remove Proteinase K and other enzymes.

ProCipitate™ provides high quality DNA suitable for automated sequencing, Southern blotting, and restriction digestion. ProCipitate™ is available as a suspension reagent and in ProPrep™ kits for specific applications and high-throughput 96 well filter formats.

Sample Size	ProCipitate™ Typical Usage
10 ml Yeast Culture	1-2 ml
Mouse Tail	250-500 µl
4 mm Plant Leaf	100-200 µl

Cell Lysis

⇩

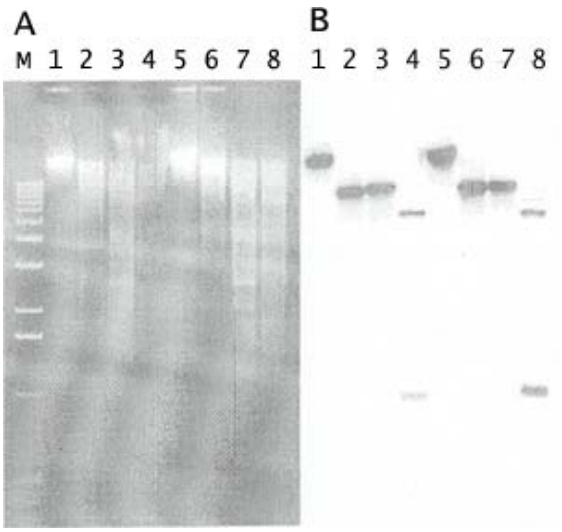
Protein Removal
(ProCipitate™ Treatment)

⇩

DNA/RNA Precipitation
(Alcohol Precipitation)

⇩

Downstream Applications
Sequencing
PCR
Micro-Arrays



Agarose gel electrophoresis and Southern blot analysis comparing genomic DNA from yeast purified by phenol/chloroform and by ProCipitate™.

A. Lanes 1-4 are isolated by traditional phenol/ chloroform methods.
Lanes 5-8 were purified using non-hazardous ProCipitate™
Lanes 1 & 5 are undigested.
The other lanes were digested with restriction enzymes as follows:
(BAM HI – lanes 2 & 4),(Eco RI – lanes 3 & 7),
(Hind III – lanes 4 & 8)

B. Southern Blot. The DNA was transferred and hybridized to a labeled probe and exposed to film for 3 hours.
Lanes 1-8 correspond to A.

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Blanket order or large quantity discounts are available for any of these items.

Product Description	Size & Quantity	Item Number	Price
ProCipitate™	30 ml	P0050-30	$335
ProCipitate™	100 ml	P0050-100	$870
ProCipitate™	50 ml	P0050-500	$3206.67
High-Throughput Genomic DNA Isolation Kits			
ProPrep™ Genomic 96	96, 50µl whole blood	PPG-96	$280
ProPrep™ Genomic 960	10x96, 50µl whole blood	PPG-960	$1980
ProPrep™ Genomic SM-50	50, 0.1ml whole blood	PBKSM-50	$210
ProPrep™ Genomic XL-2	2, 10ml whole blood	PBKXL-2	$235
ProPrep™ Genomic XL-10	10, 10ml whole blood	PBKXL-10	$665
ProPrep™ Genomic Blood Card 96	96 blood spots	PBC-96	$180
ProPrep™ Genomic Blood Card 960	10x96 blood spots	PBC-960	$1441

Product Description	Size & Quantity	DNA Yield* Per Prep	Item Number	Price
High-Throughput BAC & Plasmid DNA Isolation				
ProPrep™ BAC Mini 100	100, 2 ml cultures	2 µg BAC	PMK-100	$255
ProPrep™ BAC Mini 1000	1000, 2 ml cultures	2 µg BAC	PMK-1000	$1078
ProPrep™ BAC 96	1x96, 2 ml cultures	2 µg BAC	PLF-96	$310
ProPrep™ BAC 960	10x96, 2 ml cultures	2 µg BAC	PLF-960	1595
ProPrep™ BAC Omni 200	Up to 200 ml cultures	50 µg BAC	PPO-200	$450
ProPrep™ BAC Omni 1000	Up to 1000 ml cultures	250 µg BAC	PPO-1000	$1078
ProPrep™ Plasmid 4x96 (384 preps)	4x96, 250µl cultures	2-3 µg, ≈ 150 ng/µl	PPF-4x96	$385
ProPrep™ Plasmid 60x96 (5760 preps)	60x96, 250µl cultures	2-3 µg, ≈ 150 ng/µl	PPF-60x96	$4800

*Typical under recommended growth conditions, actual results may vary.

STORAGE

ProCipitate™ is an aqueous suspension polyelectrolyte in distilled water. Shake well before use. The reagent when not used must be kept sealed and stored at 4°C. ProCipitate™ retains full activity when stored at 4°C for about 6 months. For long term storage, please contact technical services. ProCipitate™ performs optimally in a pH range of approximately 4 to 6, however the polyelectrolyte is sufficiently acidic (pH 4) to lower the pH to within its optimal working range in most applications.

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Performance Characteristics
Typical Volume Usage

Protein	ProCipitate™: Sample	Removal
BSA, PBS @ 30 mg/ml	1 : 1	>99%
BSA, 1%SDS @ 30 mg/ml	1 : 1	>99%
BSA, 3M GuSCN @ 30 mg/ml	1 : 1	>99%
Human Serum	1 : 1	>90%
Human Serum, 1% Surfactant	1 : 1	>95%
Nucleic Acid Recovery	**ProCipitate™: Sample**	**Recovery**
Calf Thymus DNA, A_{260} = 1.00	1 : 1	>95%
Total RNA, A_{260} = 1.00	1 : 1	>99%

Application	Sample Size	ProCipitate™
Whole Blood, GuSCN Direct Lysis	50 µl	250 µl
Whole Blood, from dried blood card	7 mm punch	250 µl
Plasmid DNA, 96 well format	0.3 ml 2xYT culture	20 µl
Plasmid or BAC DNA, bulk processing	200 ml LB culture	5 ml
BAC/PAC DNA, miniprep	1.5 ml 2xYT culture	80 µl
Mouse Tail Genomic DNA	Mouse Tail	250-500 µl
Plant Genomic DNA	4 mm leaf	100-200 µl

PROTOCOL

1. Resuspend ProCipitate™ by shaking well prior to use.

2. Lyse sample to dissociate nucleic acids from histones and other proteins. Using wide bore or cut pipette tips, add the appropriate volume of ProCipitate™ to deproteinize sample. Use Table above as a guide for volume addition or try several volume ratios starting with a maximum of 1 ml ProCipitate™ to 1 ml of the sample (1 : 1 volume ratio).

3. Gently mix by inversion for 5 minutes at room temperature.

4. Centrifuge sample at 3000 x g for 15 minutes or microfuge at 16,000 x g for 5 minutes.

5. Recover purified nucleic acids contained in the supernatant.

6. Continue with alcohol precipitation or other suitable methods. Note: Buffer condition may be at a moderately acidic pH and there may be a small volume dilution.

Genomic DNA & Total RNA

ProCipitate™ can be generally substituted for phenol/chloroform in protocols recommended for genomic DNA and total RNA isolations. ProCipitate™ maintains it's reactivity in solutions containing surfactant (i.e. SDS, Triton, Tween, N-Lauroylsarcosine). It is suitable for removal of Proteinase K and ribonuclease activity. ProCipitate™ is also available as an integral component of ProPrep™ Genomic & Genomic 96 specifically for whole blood.

Viral Nucleic Acid Isolation

Reference: Schwab, K.J., De Leon, R., and Sobsey, M.D., *Concentration And Purification Of Beef Extract Mock Eluates From Water Samples For The Detection Of Enteroviruses,*

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Hepatitis A Virus, and Norwalk Virus by Reverse Transcription-PCR, Applied and Env. Microbio, 61:531-537, 1995.
Another polyelectrolyte reagent, Viraffinity™, also can be utilized. Please contact us.

ProCipitate™is Scalable
The volumetric ratio of ProCipitate™ to sample can be adjusted up or down depending on the concentration of protein in the sample. Please contact us to obtain guidance in achieving the optimal volume ratio.

Mouse Tail DNA
This protocol comes from email communication with Dr. Tom Nugent at the Scripps Research Institute in San Diego, CA: "We have routinely used this protocol in our laboratory for the last 3 years because it's a relatively simple and very reliable way to process a large number of samples."

1. Digest mouse tail tip in 0.6 ml SET buffer [1% w/v SDS, 5 mM EDTA, 10mM TRIS, 100 mM NaCl] with Proteinase K to a final concentration of 200 μg/ml (2-3 h at 50°C or overnight at 37°C). Spin down tubes. Transfer 0.5 ml into a new tube.

2. Add 0.5 ml ProCipitate™. Incubate 10 min. Mixing every 2-3 min.

3. Spin down sample. Remove 0.5 ml of supernatant.

4. Precipitate DNA with 1/10 volume NaOAc and 0.8 ml Ethanol. Spin down.

5. Dry residual ethanol and resuspend in water or TE.

References

1. U.S. Patent Numbers 5,294,681, 5,453,493 and other patents pending.

2. U.S. Patent Number 5,538,870, Method for Preparing Nucleic Acids For Analysis And Kits Useful Therefore.

3. Transgenic labeling of hair cells in the zebrafish acousticolateralis system Brian M. McDermott Jr Gene Expression Patterns Volume 10, Issues 2-3, February-March 2010, Pages 113-118

4. Formation of Deoxyguanosine Cross-Links from Calf Thymus DNA Treated with Acrolein and 4-Hydroxy-2-nonenal. Ivan D. Kozekov, Robert J. Turesky, Guillermo R. Alas, Constance M. Harris, Thomas M. Harris, Carmelo J. Rizzo Chemical Research in Toxicology 2010 23 (11), 1701-1713

5. Genome-wide sequence and functional analysis of early replicating DNA in normal human fibroblastsStephanie M Cohen, Terrence S Furey, Norman A Doggett, and David G Kaufman BMC Genomics. 2006; 7: 301. Published online 2006 November 29. doi: 10.1186/1471-2164-7-301.

6. Dr. Domon, National Agricultural Research Center for Kyushu Okinawa Region, Japan, Extraction of Rush DNA, unpublished personal correspondence, 2004.

7. Krupey, J., et al, 100,000+ PCRs Possible from 10 ml Blood, poster Biotechniques Symposium, 2003.

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CONTACT US

We welcome your questions and comments regarding our products.

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