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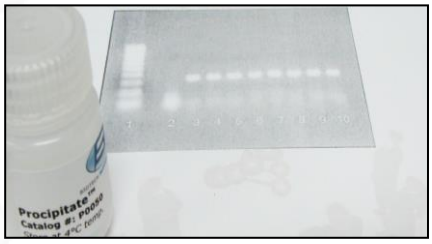
ProCipitate™

Superior Substitute to Phenol/Chloroform for DNA Isolation & Protein Binding

- Removes protein contaminants & leaves DNA soluble and unreacted
- Ideal for applications when the alternative kits don't fit, or are not optimal
- Adaptable to any sample size and can be automated
- Pathogen and infectious disease testing
- Tissue and paraffin-embedded tissues



The **ProCipitate**™ strategy is opposite to common prep strategies, as instead of binding nucleic acids, the protein is efficiently depleted with no interaction with the soluble nucleic acids.

ProCipitate™ is a unique protein binding reagent developed from patented solid-phase polyelectrolytes. These elastomeric polymer suspension reagents are prepared in an extended state due to strong electrostatic repulsion of the repeating polymeric acid groups. Upon interaction with proteins, salt bridges form and the resultant complex collapses to a lower energy state, expelling water much like dehydration processes taking place within solvent precipitations. Consequently, aggregation of proteins is strongly promoted and occurs even in high ionic strength or surfactant containing solutions. Most importantly, nucleic acids remain unreacted and are quantitatively recovered in solution.

In this way, **ProCipitate**™ is characteristic of phenol/chloroform separation, a long established benchmark for nucleic acid isolation. However, **ProCipitate**™ is non-volatile, non-hazardous, and has the additional benefits of solid-phase suspensions; that is - the adaptability to filtration and automation. **ProCipitate**™ and related **ProPrep**™ kit products have been on the market for close to 20 years being used throughout the Human Genome Sequencing Project. It is routinely used for improving the yield consistency and protein depleted quality of DNA. Such improvements have been cited in sequence and PCR quality for a variety of applications, most notably in the template preparation of large insert plasmids (cosmids & BACs) and PCR suitability for infectious agents from large volumes and from paraffin-embedded tissues.

ProCipitate™ can even be used for enrichment of other macromolecules including viruses, proteoglycans, polysaccharides, glycolipids, and highly substituted polymer conjugates (i.e., PEG), which serve to mask salt bridge formation and retain solubility. A full list of references is provided at the end of this product sheet.

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Product	Size	Item No.
ProCipitate™	30 ml	P0050-30
ProCipitate™	100 ml	P0050-100

ProCipitate™ and related products can be customized to fit specific needs. It also can be supplied in bulk quantities. Please contact our sales office or any of our worldwide distributors for more information.

Cell Lysis & Nucleosome complex disruption

↓

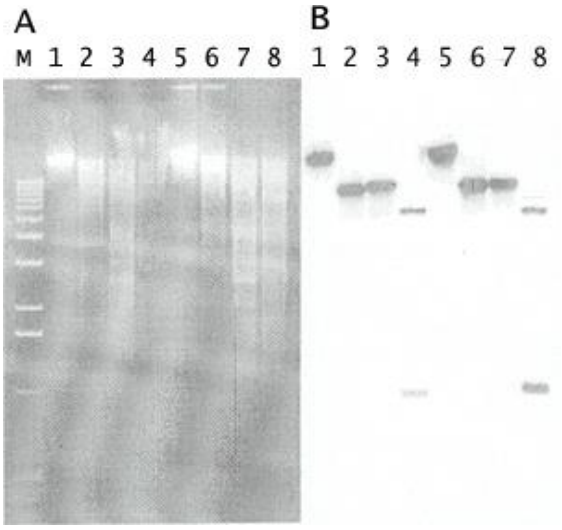
ProCipitate™ treatment
(Protein Extraction)

↓

DNA/RNA Final Preparation
(Alcohol Precipitation)
Or
(Dilution) or
(Inhibitor Neutralization)

↓

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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Considerations for optimal use

The optimal use of **ProCipitate™** for nucleic acid isolation should consider these 4 necessary processes:

1. The cells/tissue must be sufficiently lysed so that the intra-cellular fraction is released into the surrounding media, and the nucleosome (histone protein/ DNA complex) is efficiently disrupted.

2. The amount of **ProCipitate™** required will depend on the starting sample protein load, guidelines f0or which are provided below, and

3. The soluble DNA upon treatment, must be finally prepared to neutralize any inhibitory effects of the lysis condition. Typically, this is done by alcohol precipitation, dilution, or chemical neutralization; for which some of these protocols are documented in the references provided. Because **ProCipitate™** is reactive under diverse lysis conditions, the user has great latitude in designing a protocol optimized for their own particular application.

4. **ProCipitate™** reactivity is indistinguishable between DNA and RNA.

Sample Size	ProCipitate™ Typical Usage
1 ml Yeast Culture Genomic DNA	200 µl
Mouse Tail Genomic DNA	200 µl
1 mm Plant Leaf	50 µl
2.0 ml culture BAC Preps	80 µl
5µm paraffin-embedded tissue	200 µl
250 µl culture Plasmid Preps	20 µl
200 ml Large Scale BAC Preps	5 ml
Dried Blood Card or ~ 40 µl Whole Blood	400 µl
200µl lysed cell pellet	200 µl

ProCipitate™ performs optimally in a final pH range of approximately 4 to 6, however the polyelectrolyte is sufficiently acidic (pH 4) to lower the final reaction pH to within its optimal working range in most applications.

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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.

Performance Characteristics

Protein	ProCipitate™: Sample	Removal
BSA, PBS @ 30 mg/ml	1 : 1	>99%
Human Serum	2 : 1	>90%
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%

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.

ProCipitate™ is Scaleable

The volumetric ratio of **ProCipitate™** to sample can be adjusted up or down depending on the concentration of protein in the sample. Once established, these same ratios can be used to process volumes at any scale.

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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, 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.

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

ProCipitate™ appears in several articles and books on Food Safety

- **Foodborne Disease Handbook,** Second Edition,: Volume 2: Viruses: Parasites
  By Y. H. Hui, Sayed A. Sattar, Wai-Kit Nip
  "Viruses in the PEG eluants were precipitated…by an equal volume of ProCipitate™."

- **Health-related Water Microbiology,** Volume 27, Issues 3-4, Pergamon, 1993
  "ProCipitate™ was an effective method to purify the sample and dramatically improve virus detectability by RT-PCR."

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Patents

Composition and utility patents for ProCipitate™ and related technologies are covered under U.S. Patents Numbers 5,294,681, 5,453,493 & 5,658,779.

U.S. Patent Number 5,538,870, *Method for Preparing Nucleic Acids For Analysis And Kits Useful Therefore*. This patent shows the beneficial effects of ProCipitate™ in protocols which neutralize SDS with non-ionic detergents, are PCR compatible, and require no alcohol precipitation.

Plasmids, Cosmids, BACs

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Food Safety, Enteric Viruses and Environmental Sampling

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CONTACT US

We welcome your questions and comments regarding our products.

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