GE Healthcare

illustra
CsTFA
for isolation and separation of nucleic acids by isopycnic centrifugation

Product booklet

Code: 17-0847-02

Page finder

1. Legal

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http://www.gehealthcare.com/lifesciences

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only*.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store the CsTFA reagent at 2–8°C tightly sealed in its original container. Density changes will be minimized if CsTFA solutions are withdrawn by a syringe needle through the septum closure. Since solutions stored for long periods in open containers, or transferred to other containers may change slightly in density, CsTFA solutions should be routinely checked to confirm density.

2.3. Expiry

For expiry date please refer to outer packaging label.

3. Components


Do not use cellulose nitrate centrifuge tubes when preparing the gradient


Do not use cellulose nitrate filters when sterilizing

CsTFA is an aqueous solution of Cesium Trifluoroacetate with a density of 2.0 g/ml ± 0.05. The exact density of each lot is indicated on the label. CsTFA contains approximately 134 g of Cesium Trifluoroacetate per 100 ml of solution.
CsTFA is supplied at approximately neutral pH, free of preservatives and buffers.

4. Description

4.1 The basic principle

CsTFA is a standardized solution of Cesium Trifluoroacetate intended for the isolation and separation of nucleic acids by isopycnic centrifugation. CsTFA is used in a fashion similar to Cesium Chloride (CsCl) and Cesium Sulphate (Cs_2SO_4). The trifluoroacetate ion, however, imparts properties to CsTFA that result in higher quality nucleic acid preparations when compared to traditional Cesium density gradient media.

CsTFA is distinctive in that it:

- Isopycnically bands all types of RNA
- Solubilizes and denatures proteins, resulting in their removal from nucleic acids.
- Inhibits nuclease activity resulting in undegraded DNA and RNA.

Technical specifications for CsTFA are listed in Table 4.1. Table 4.2 compares physical properties of CsTFA and CsCl.

Table 4.1. Specifications for CsTFA (4)

Parameter	Value
Density	2.0 ± 0.05 g/ml
Purity of cation	>99.99% Cs
Purity of anion	>99% TFA
Total heavy metal ion content	<12 ppm
pH	4–9 (unbuffered)
Ultraviolet absorption*	A_{260} <0.3
(2.0 g/ml solution)	A_{280} <0.075

*See Figure 3 for a typical absorption spectrum of undiluted CsTFA.

Table 4.2. Physical properties of CsTFA vs CsCl

Property		**CsTFA**	**CsCl (1)**
Maximum density	(g/ml)	~2.6	1.9
Maximum solubility	(g/ml)	2.5	1.23
	(g/g)	89%	60%
Maximum molarity	(M)	~10	7.36
Formula weight (g)	245.93	168.37	

5. Protocol

Metal surfaces or instrument parts exposed to CsTFA should be rinsed thoroughly with water or alcohol immediately after use. Skin exposed to CsTFA should be washed with soap and water. There is no evidence that CsTFA is significantly more toxic than other Cesium salts.

5.1. Preparation of sample

CsTFA solubilizes and denatures proteins, assisting in their removal from nucleic acids during isopycnic centrifugation. This property reduces and may even eliminate the need for prior deproteinization.

- Samples relatively low in protein content such as viruses and ribosomes usually do not require prior deproteinization.
- Samples containing high amounts of protein should be partially deproteinized prior to centrifugation using conventional purification techniques such as phenol extraction or protease treatment.
- Samples particularly adversely susceptible to phenol extraction are good candidates for direct application onto CsTFA gradients.

CsTFA inhibits nuclease activity during centrifugation (2). Nuclease inhibitors should be present, however, during sample preparation to minimize nuclease activity prior to CsTFA centrifugation.

Sodium Dodecyl Sulphate (SDS) forms insoluble salts in the presence of Cesium and should be removed prior to sample application. An alternative detergent compatible with CsTFA is Sodium N-Lauroyl Sarcosinate.

5.2. Estimation of nucleic acid density

CsTFA hydrates nucleic acids causing them to band at lower densities than in CsCl. It is therefore important to estimate the density of the nucleic acid in CsTFA for correct gradient preparation.

Most double-stranded DNAs band at densities between 1.60–1.63 g/ml in CsTFA. A closer density estimate is possible if the percent (G+C) or the density in CsCl is known. An approximate estimate of the density in CsTFA can be made from Figure 5.1. Single-stranded DNA bands in CsTFA at a density approximately 0.08 g/ml greater than the corresponding native DNA.

Banding densities of RNAs are influenced by the degree of secondary structure. RNAs with a high degree of secondary structure, such as ribosomal RNA and transfer RNA, normally band near 1.65 g/ml in CsTFA. RNAs with little secondary structure, such as messenger RNA, will typically band at densities around 1.90 g/ml.

Proteins are found in the density range 1.20–1.50 g/ml in CsTFA.

5.3. Preparation of gradient


Do not use cellulose nitrate tubes


Temperature, the use of denaturants or organic solvents, and extremes of pH may greatly affect the resistance of the tube material. The chemical resistance should be confirmed under actual running conditions.

1. The chemical resistance of centrifuge tubes varies according to the material used. Tube materials such as polyallomer, polyethylene, polypropylene, polycarbonate and glass are generally satisfactory.
2. CsTFA should be diluted with buffers normally used for storing and maintaining nucleic acids.
   Buffers containing EDTA, pH 7–8 are recommended. A typical buffer to use is 0.1 M Tris HCl, pH 8.0; 0.1 M KCl, 1 mM EDTA.
3. The following formula is used to calculate the volumes required to obtain a solution of desired density.

$V_x = V_o (\rho_o - \rho)/(\rho - \rho_x)$

Where V_x = volume of diluting medium in ml

V_o = volume of original CsTFA solution in ml

ρ_o = density of CsTFA in g/ml (see bottle label)

ρ_x = density of diluting medium (water =0.998g/ml at 25°C)

ρ = density of desired solution produced in g/ml

Example: To obtain a desired density of 1.600 g/ml, add 0.664 ml water to 1 ml of CsTFA (ρ_o = 2.00 g/ml).

CsTFA, supplied at a predetermined density, is diluted to the required density. For very precise work the density of the diluted solution should be checked and readjusted before use (see section 6).

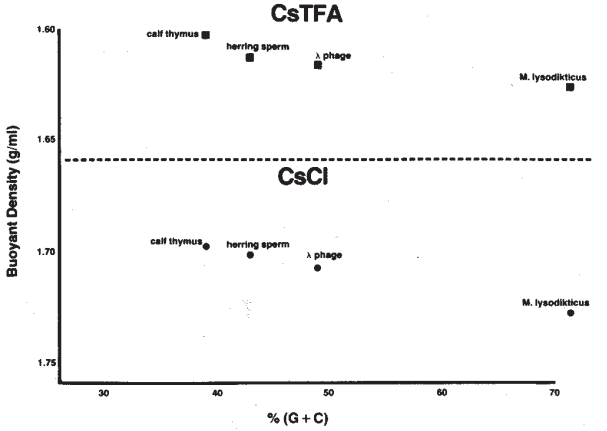

Figure 5.1. Relationship between % (G+C) of various DNAs and their buoyant densities in CsCl and CsTFA. DNA samples were centrifuged in CsTFA gradients at 40 000 rpm (g_{av} = 190 000) for 36 hours at 18 °C in an International No. 482 Angle-head Rotor. Values for DNA banding in CsCl at 25 °C were obtained from the Handbook of Biochemistry, Second Edition (1).

4. CsTFA gradients are prepared using the same methods established for CsCl gradient formation (3). By diluting CsTFA to the estimated nucleic acid density and subjecting the solution to high *g*-forces, CsTFA will self-generate a gradient capable of isopycnically banding the nucleic acid of interest.
5. CsTFA exhibits bacteriostatic and bacteriocidal properties such that sterilization is not generally necessary. If desired, CsTFA can be sterilized by sterile filtration or autoclaving. Do not use cellulose nitrate filters. Filters made of mixed esters of cellulose are acceptable. Since autoclaving may alter the density of CsTFA solutions, the density should be reconfirmed post-autoclaving.

5.4. Application of sample

Sample application techniques are the same as for CsCl (3). Samples are most conveniently combined with the entire CsTFA solution (see section 6). Diluting the sample in this manner enhances the effectiveness of CsTFA in solubilizing proteins and inhibiting nuclease activity.

5.5. Ultra centrifugation conditions

Recommended running conditions for CsTFA are very similar to those for CsCl. CsTFA forms steeper gradients than CsCl when run under identical conditions. These gradients permit isopycnic banding of nucleic acids which differ greatly in density (i.e., DNA and mRNA).

CsTFA gradients may be generated in vertical, fixed-angle and swinging-bucket rotors. If desired, the gradient steepness may be altered by changing to a rotor of different geometry. Fixed-angle and vertical rotors produce shallower gradients than swinging-bucket rotors (3) and are recommended for increasing the resolving power of CsTFA gradients during isopycnic centrifugation.

Typical centrifugation conditions are 100 000 x g_{av} for 36–72 hours.

Running temperatures of 4–25 °C are recommended. Deproteinization is more effective at higher running temperatures. The buoyant density of nucleic acids varies only slightly with temperature unless temperatures approach the melting point of the nucleic acid.

It is the responsibility of the user to ensure that the centrifuge rotor is operated within the design limits of the machine. Most manufacturers recommend that the derating factor be used whenever the average gradient density exceeds 1.2 g/ml.

5.6. Fractionation of gradients

CsTFA gradients can be fractionated by any of the common methods used for Cesium salts. For gradients containing large amounts of protein, it is recommended that fractionation originates from the bottom of the tube. This avoids the possibility of contaminating the nucleic acid with protein.

5.7. Determination of density

Densities of CsTFA solutions after fractionation can be determined using any method applicable to Cesium salts. These methods include use of calibrated micropipettes, densitometry, conductivity determination and refractive index measurements. The most convenient method is by measuring the refractive index.

After fractionation the refractive index of CsTFA solutions should be measured as soon as possible since they are susceptible to changes in density on standing. Using Figure 2 the density of CsTFA can be determined. Alternatively the following equation can be used.

$$\rho^{25} = 163.559 - 262.271\,(n_D^{25}) + 105.281\,(n_D^{25})^2$$

Where ρ^{25} = density of CsTFA at 25 °C

n_D^{25} = refractive index of CsTFA at 25 °C

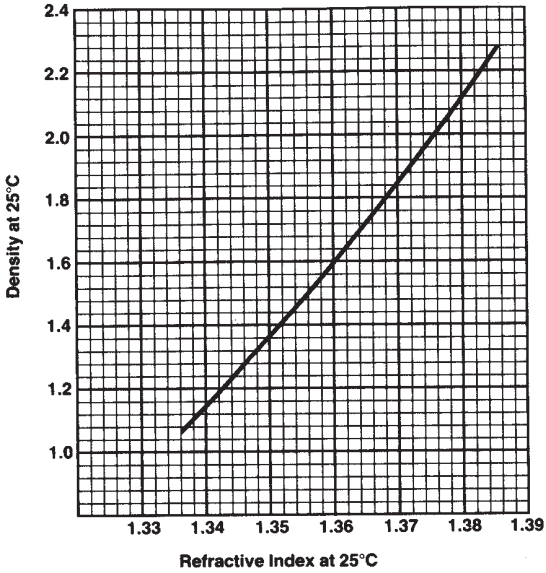

Figure 5.2. Relationship between Refractive Index and Density for CsTFA

5.8. Determination and recovery of nucleic acids

CsTFA possesses a low degree of absorbance in the ultraviolet region thus facilitating detection of nucleic acids (Figure 5.3). Fractions from CsTFA gradients may be monitored spectrophotometrically at 260 nm.

Nucleic acids may be separated from CsTFA by traditional techniques such as gel permeation chromatography, ethanol precipitation and dialysis. Unlike CsCl, CsTFA is freely soluble in ethanol so that co-precipitation with nucleic acids cannot occur.

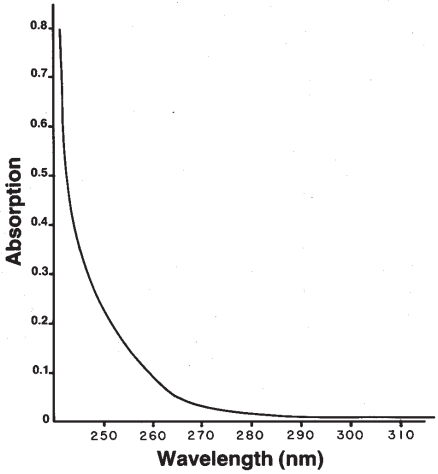

Figure 5.3. CsTFA does not interfere with the intercalation of dyes such as Ethidium Bromide to DNA. However, since CsTFA is soluble in n-Butanol and Isopropanol, these commonly used solvents should not be used for the removal of Ethidium Bromide from DNA subsequent to centrifugation. Alternatively ethanol precipitation can be used, which removes most of the dye. If further purification is required, an ion exchange column such as DEAE-Sephacel™ can be used.

6. Additional information

6.1. References

1. Handbook of Biochemistry, Sober, M.A. (Ed.), Chemical Rubber Company, Cleveland, Ohio, (1970).

2. Carter, C. *et al*., *BioTechniques*, pp.142–146, (1983).

3. Chambers, J.A.A and Rickwood, D. in: Rickwood, D. (Ed.), *Centrifugation: A Practical Approach,* Information Retrieval Ltd., London and Washington, D.C., pp. 119–133, (1978)

6.2. Related products

RNAspin Mini RNA Isolation Kit	25-0500-70
RNAspin Midi RNA Isolation Kit	25-0500-73
RNAspin 96 RNA Isolation Kit	25-0500-74
QuickPrep™ *Micro* mRNA Purification Kit	27-9255-01
QuickPrep mRNA Purification Kit	27-9254-01
mRNA Purification Kit	27-9258-01
Oligo (dT)-Cellulose Type 7	27-5543-02

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