P09E

■ Buffering agents and Buffers

Introduction

Interchim provides high quality biochemicals for use in Biotech, Purification, Molecular Biology, and Cell Culture.

See other catalogues of biochemicals (by substances types, applications...),

biochemical list by alphabetic order or on line search in all Interchim supply.

See technical quality grades

Most applications in biotechnologies and biochemistry of proteins operate in aqueous solutions. Other more or less polar solvents are however useful, and apolar organic solvents are used to solubilize reagents in typically in biochemistry.

Water is determinant to interactions of biological systems, dissociating in H+ and OH- ions thus interfering with ions and charged biomolecules, but also interacting by Van de Wahl binding to solvate biomolecules and by hydrophobic interactions to form micelles or precipitates...

To that point, **buffers** are aqueous solutions containing partly neutralized weak acids or bases that show little change in pH (H+ concentration) whatever ions are added. Requirements should be considered for the choice of the buffer, and it's use in each application. I.e. the pH should be determined at the final temperature, in presence of salts (i.e. phosphate pH change with salts concentration) near the pKa of the buffering compound. The buffering compound should not absorb at wavelengths (i.e. at 240-270nm for mass spectrometry)…

Classic used buffering agents are **mineral buffers** (Phosphate, Tris, Borate, Citrate, Glycine...), but also several **organic buffers** (Glycine, DEA,...). Many requirements should be met (buffering range, solubility, compatibility with spectrometric or immunométric or cell assays,...) and several other points considered such as habits, availability and price. As a result, standard buffer, and even any choosen buffer are often not ideal at one point or the other, and one might take benefits from more specific and new buffers.

Biological buffers differ from classic mineral ones to several points: they have pKa values closer to physiological pH (between 6 and 8). These buffers are not toxic to cells, and are not absorbed through cell membranes. The concentration, temperature, and ionic composition of the medium has minimal effect on the buffering capacity. They are resistant to enzymatic and non-enzymatic degradation, furthermore they are essentially transparent to visible and ultraviolet light.

For solubilizing, many reagents require a more polar (i.e. Ethanol) or apolar solvant (DMSO, DMF). In biochemistry and chromatography applications, organic solvants are used to create more or less or gradient of polarity (Acetonitrile, Propanol,...) while acidic or alkaline subtances are require to create desired pH (Citric acid, TFA,...). As several most are *nocive* and *even toxic*, requiring often *specific operating conditions*, on should consider <u>Green Substitutes or Alternatives</u>^(O).

Examples include limonène, (xylène->) histochoice, ...

standard buffering components

Solvants

Water | DMSO, DMF (solubilisation) More Solvants

• Buffering agents, and their formulated buffers

Borate | Citrate | Glycine | Tris | Phosphate | ... More classic mineral and organic buffering agents/buffers

Hepes | Mops | ... More biological buffers (Good's buffers)

• Additives for buffer solutions preparation

•Formulated buffers for electrophoresis/blotting TBE, TAE, TG, TG-SD, TTE...: see Tris formulated buffers

for chromatography Glycine 1M, Tris Ph3, TEA,...

for Cell culture and assay: PBS, Dulbecco's PBS, Hepes, DMSO,...

Solvants

*Water and polar solvants

Water Nuclease free, Sterile, RNase-Free Solution 457420 500 ml

Ethanol

*Organic solvants

DMSO, ACS grade 36765A, 500ml

DMF

See <u>Green Substitutes and Alternatives</u>⁰

See also solvents for chromatography, purification,... (i.e. TEA, TFA,...)

Buffers

See also buffers by applications: Immunodetection: Buffers⁽⁾, Saturating agents⁽⁾, Enzymatic substrates⁽⁾

Biochemistry: <u>Buffers for electrophoresis</u>⁰, ... **Cell Culture**: Cell culture media components

Buffer descriptions are blow by buffering agents with their formulated buffers, as powder, solution, concentrates,...:

Go to: Borate Citrate Glycine Tris Phosphate Others

Borate

 $pK_{a1}(25^{\circ}C)=9.24$ | pH range 8.5-10.2

Borate buffered saline should not be used in the presence of polyols, including carbohydrates and their derivatives with which they may chelate compounds. Borate buffers also have a high bacteriocidal effect. The use of borate buffers in gel electrophoresis of proteins can result in spreading of the zones.

Boric Acid UP070440 1 kg

 $MW:61.83\ pKa1-9.24,\ pKa2-12.74,\ pKa3-13.80,\ pH\ range:\ 8.5-10.2$

Boric Acid Proteomics Grade 10853A 500 g 10853B 1 Kg

Citrate

pKa1 - 3.13 2.2-6.5, pKa2 - 4.76 3.0-6.2, pKa3 - 6.40 5.5-7.2 | pH range 3-6.2

Citrate is used notably for elution in affinity chromatography, but also for cell media.

Citric Acid UP168781 1 Kg MW:192.1

Citric Acid ACS grade/ Biotech grade 673410 500 g

MW:192.1

Citric Acid, Trisodium Dihydrate 218830 1 Kg 218831 2.5 Kg

IW:294.

Citric Acid, Trisodium Dihydrate Proteomics Grade 10853A 500 g 10853B 1 Kg

IW:294.1

Citric Acid, Ammonium Salt, Dibasic, UltraPure N12630 500 g N12631 1 Kg

MW:226.2

Glycine

pKa1 - 2.35 pH range: 2.2-3.6, pKa2 - 9.78 | pH range: 8.2-10.6

Glycine is used in buffers notably for elution in affinity chromatography, in electrophoresis buffers, but also as quenching agent biochemistry.

Glycine UP018225, 1Kg

MW:75.07

TG buffer (Tris/Glycine) see Tris buffers
TG-SDS buffer (Tris/Glycine/SDS) see Tris buffers



Tris, and formulated Tris buffers

Syn.: TRIS; Tromethane; Tromethamine, 2-Amino-2-hydroxymethyl-1 3-propanediol, Tris(hydroxymethyl) aminomethane

pK_a(25°C)=8.30 ; Useful | pH range 7.2-9.0

Tris buffers are preferable over phosphate buffers to avoid complex formation with ionic species such as calcium and magnesium in blood. It generally does not suit biochemistry applications because it contains primary amine (interferes with amine reactive agents) and it's appreciable solubility in organic solvents.

Tris (base)	UP031658 500 g	UP031657 1Kg

UP031657 5Kg

Tech sheet

Tech sheet

Tris HCI UP09154D 500 g UP09154E 1 kg

MW: ; Tech sheet UP09154F 5x1 kg

Tris buffer 0.1M solution pH 7.4 nuclease free biotechnology grade 587550 500 ml 587551 100 ml

Tris buffer 0.5M solution pH 6.8 biotechnology grade 725200 500 ml

Tris buffer 0.5M solution pH 6.8 proteomics grade 725201 500 ml

Tris buffer 1.0M solution pH 7.5 sterile ultra pure grade N13710 100 ml

Tris buffer 1.0M solution pH 8 sterile biotechnology grade 586780 100 ml 586781 500 ml

Tris buffer 1.0M solution pH 9 sterile ultra pure grade N13720 250 ml

Tris buffer 1.0M solution pH 10 sterile ultra pure grade N13740 250 ml N13740 250 ml

Tris buffer 2.0M solution pH 7.5 N14620 1 L
Tris buffer 2.0M solution pH 7.8 N14610 500 ml

 TAE Powder
 892580 1 u (40 L)

 TAE Ready-pack
 665100 2 packs (50 L)

 TAE Solution 25X Concentrate
 UP892574 1.6 L

 TBE Powder
 892533 1 u (40 L)

 TBE Ready-pack
 892535 2 packs (20 L)

 TBE Solution 5X Concentrate
 N14790 1 L N14791 4 L

 TBE Solution 10X Concentrate
 UP86510A 5 L UP86510C 4 x 5 L

TBE disodium Ready-pack 473840 2 packs (20 L)

Tris Buffered Saline (TBS), 20X Liquid Concentrate N14580 4 L Tris Buffered Saline (TBS), 20X Ready-PackTM 740040 2 packs

TTE Solution 10X concentrate R59980 1 L R59981 5 L TTE Ready-pack R59982 1 Pack (10 L)

Phosphate, and formulated Phosphatebuffers

Phosphate Buffered solutions, and notable saline ones (PBS: typical composition is NaCl 100mM, HPO4 20mM, pH 7.4) are widely used, to dilute and incubate or wash reagents in immunodetection techniques, for cells in cell culture et cell assays, in Biochemistry notably with proteins...

Phosphate buffer should not be used in assays where competition for phosphate groups, or complex formation with a metal ion is essential for the enzyme activation. Phosphate ions will inhibit carboxypeptidase, carboxylase, urease, muscle diaminase, formase and phosphoglucomutase.

PBS Ultrapure, powder packs

UP68723A 1pack (10 L) 68723B, 10x1L pack

Tech sheet

PBS 1X Solution

PBS Sterile (0.2μm filtered, autoclaved)

N13522, 500ml

PBS Concentrate 10X

N14012, 1L

PBS Concentrate 20X

N1376A, 500ml

Tech sheet

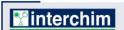
Tech sheet

PBS Ultrapure, ready-to-use tabs

UP307157 100 tabs (100ml)

PBS with Tween 20, pH 7.5 N13810 500 ml N13811 1 L

Dubelco PBS



Other mineral and organic buffers

HEPES, free acid UP061940, 250 g 06194P 1, Kg

MW:238.3 pKa:7.55 | pH range: 6.8-8.2

MOPS, UltraPure UP062000, 100 g UP062002, 500 g

MW: 209 pKa: 3 7.20 | pH range: 6.5-7.9

• See more Good's buffer in the technical sheet FT-062000 (pka 6 to 10; as powder, free of salts forms, solutions).

Imidazole

Imidazole 020220, 10 g 020228, 50 g

MW:68.08 pKa: 6.95, pH range 6.2-7.8

Imidazole Proteomics grade BI9270, 10 g BI9271, 50 g

· Succinic Acid

Succinic Acid Free Acid N12170, 500 g N12171, 2.5 Kg

MW: 118.09 pKa1 - 4.21 3.2-5.2, pKa2 - 5.64 5.5-6.5

Additives for buffers preparation

(short list with link to the catalog for more products and information)

*Detergents, Chaotropes,.

SDS, powder UP649100 500 g

SDS, 20 % solution UP896826 500 ml UP896827 2x500 ml

Urea, Molecular Biology Grade UP031903, 500g UP031904, 1Kg UP031909, 5Kg

Urea, 8 M Solution N13830 250 ml Urea, 8M solution, proteomics grade N13831 250 ml

See the catalog for more **Detergents**⁽⁾ with detailed information.

See also the section 'Protein extraction' (detergents powder, solution and formulated for extraction procedures)

*Antibiotics, Bacteriostatics

Azide, sodium salt 081125, 25g

Azide, sodium 1% solution DY8950, 100ml <u>Tech sheet</u>

Azide, sodium 10% solution NJK63A -

See the catalog for more **Antibiotics/Bacteriostatics**^{()(PW)} with detailed information.

*Protease inhibitors

Protease Inhibitor COCKTAILS

PROTEASE INHIBITOR COCKTAIL I (General use)	WT0900	Tech sheet
PROTEASE INHIBITOR COCKTAIL I (General use), Animal-Free	WT0940	Tech sheet
PROTEASE INHIBITOR COCKTAIL VI (General Use - Broad Range)	WT8220	Tech sheet
PROTEASE INHIBITOR COCKTAIL II, for Bacteria	WT8260	Tech sheet
PROTEASE INHIBITOR COCKTAIL III (for Mammalian)	WT0850	Tech sheet
PROTEASE INHIBITOR COCKTAIL III (for Mammalian) Animal-free	WT0920	Tech sheet
PROTEASE INHIBITOR COCKTAIL III (for Mammalian) Animal-free, Solvent-free	WT0890	Tech sheet
PROTEASE INHIBITOR COCKTAIL IV (for Fungi & Yeast)	WT0930	Tech sheet
PROTEASE INHIBITOR COCKTAIL V (for Mammalian) EDTA-free	WT8280	Tech sheet
PROTEASE INHIBITOR COCKTAIL VI (for Plant)	WT0870	Tech sheet
PROTEASE INHIBITOR COCKTAIL V (for Serine/Cysteine not Metallo Protease) EDTA-Free Animal-Free	WT0860	Tech sheet
PROTEASE INHIBITOR COCKTAIL VII (for broad range cysteine proteases)	DZ0280	Tech sheet
PROTEASE INHIBITOR COCKTAIL VII (for His Tagged Proteins)	WT0880	Tech sheet
PROTEASE INHIBITOR COCKTAIL VII (for His-Tagged proteins) DMSO-free	WT0910	Tech sheet
PROTEASE INHIBITOR COCKTAIL I (for Serine proteases)	WT8230	Tech sheet

Protease Inhibitor POWDERS

E64 (Cysteine Proteases Irrevers. Inhibitor) 789581 <u>Tech sheet</u>
AEBSF UP401071, 1g UP401074, 10g <u>Tech sheet</u>

See the catalog for more **Protease Inhibitors**((PW)) with detailed information.



*Saturating agents

BSA (Bovine Serum Albumine)

BSA, powder (Fraction V, Standard) UPQ4170, 100g UPG4171, 500g UPQ4172, 1Kg

Techn sheet

BSA 30% solution UP900100, 50ml UP900101, 500ml UP900102, 1L

Techn sheet. Also available as DNAse/RNAse Free grade (610531)

See the catalog for more Saturating agents ((PW)) with detailed information.

*Miscellaneous

DTT (1,4-Dithiothreitol) Biotechnology Grade UP284250 1 g UP284255 5 g

Sucrose UP252031 1 kg Sucrose, Ultra Pure Grade UP031904 1 kg

Cell culture media components

See detailed information of Cell Culture media ((PW)

Bioactive compounds

See the **Bioactive compounds**^{()(PW)}(Peptides, Proteins, Enzymes, Extract&Lysates,...)

Annexes

Definition of biochemicals quality grades

ACS: **ACS Grade**:

Materials conforming with the specifications and procedures outlined in American Chemical Society specifications

ANG: Analytical Grade.

Designates reagents suitable for use in analytical procedures.

BTG: **Biotechnology Grade**. Materials equivalent to Ultra Pure, but particularly suitable for use in Molecular biology applications. Tested for specific contaminants such as nucleases and bacteria where appropriate.

CERT **Certified/certifiable**: Materials, typically dyes and stains, that meet the requirements of the biological stain commission. Certified reagents have been tested and validated by biological stain commission.

FPG: FluoPure grade:

High quality reagents for enhanced results of critical applications in fluorescent or luminescent techniques

HPG: **High Purity grade**.

Materials of superior quality where there are no publishing standard

PRG: **Proteomics Grade**.

Materials conforming to the requirements of protein research which are tested to be nuclease, DNase and/or Protease free where applicable. Appropriate for use in Proteomics research applications.

RGG: Reagent Grade.

High Purity materials which suits most standard labs applications.

UPG: Ultra Pure grade.

Material with a purity level exceeding the various monograph grades

USP: **USP Grade**:

Materials conforming with the specifications and procedures outlined in the United States Pharmacopeia (standards for food ingredients and dietary supplements)

Related products lines

Interbiotec - BioSciences innovation – proposes a complete range of products for protein biochemistry.

• General use Biochemicals (buffers, salts, detergents, antibiotics, protease inhibitors,...) (PH



• <u>Desalting tools</u> – CelluSep tubings, SpectraPor tubings, GebaFlex, FloatALyser, SlideALyser,...

Products HighLights Overview

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