Store at 4°C

# CellSimple™ Cleaved Caspase-3 and Phospho-Histone H3 Antibody Assay Kit



Support: +1-978-867-2388 (U.S.) www.cellsignal.com/support

> Orders: 877-616-2355 (U.S.) orders@cellsignal.com

1 Kit (50 assays)

Rev. 12/07/16

# For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Storage Temp.
Cleaved Caspase-3 (Asp175)(D3E9) RabbitmAb (PE Conjugate)	12768	1 x 25 µl	4°C
Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate)	3465	1 x 25 µl	4°C
16% Formaldehyde, Methanol-free	12606	2 x 10 ml	RT
Phosphate Buffered Saline (PBS-20X)	9808	1 x 25 ml	RT

### **Species Cross-Reactivity: H**

Description: CellSimple™ Cleaved Caspase-3 and Phospho-Histone H3 Antibody Assay Kit is a fluorescent assay designed for use with the CellSimple™ Cell Analyzer. The kit includes Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (PE Conjugate) #12768 and Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (Alexa Fluor® 488) #3465. The kit allows for the quick and easy assessment of the levels of mitotic cells undergoing apoptosis.

### Specificity/Sensitivity: Antibodies provided in the CellSimple™ Cleaved Caspase-3 and Phospho-Histone H3 Antibody Assay Kit detect endogenous levels of their respective target.

Background: Caspase-3 (CPP-32, Apoptain, Yama, SCA-1) is a critical executioner of apoptosis, as it is either partially or totally responsible for the proteolytic cleavage of many key proteins, such as the nuclear enzyme poly (ADP-ribose) polymerase (PARP) (1). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 fragments. Cleavage of caspase-3 requires the aspartic acid residue at the P1 position (2). Modulation of chromatin structure plays an important role in the regulation of transcription in eukaryotes. The nucleosome, made up of DNA wound around eight core histone proteins (two each of H2A, H2B, H3, and H4), is the primary building block of chromatin (3). The amino-terminal tails of core histones undergo various post-translational modifications, including acetylation, phosphorylation, methylation, and ubiquitination (4-6). These modifications occur in response to various stimuli and have a direct effect on the accessibility of chromatin to transcription factors and, therefore, gene expression (7). Phosphorylation at Ser10 of histone H3 is tightly correlated with chromosome condensation during both mitosis and meiosis (8).

**CellSimple™ Cell Analysis System:** The CellSimple™ Cell Analyzer is a benchtop instrument that utilizes a disposable thin-film cassette and a combination of a 488 nm laser, two photomultiplier tubes (525/45 nm and 561 nm LP filters), Coulter Principle-based cell measurements, and on-board software to provide easy-to-run applications and data analysis. Data acquisition occurs within approximately 10 seconds per test. The instrument relies on disposable cassettes for sample handling, which alleviates the need for flow cell cleaning and fluidics maintenance and the instrument is small enough to be portable between the lab bench and the hood. Applications include quantitative assessments of cell viability, apoptosis, other labeled antibody markers and single and multiplexed bead-based assays for protein and cellular analysis. **Storage:** The conjugated antibodies in this kit are supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. Store at 4°C. *Do not aliquot the antibodies. Protect from light. Do not freeze.* The remaining components of the kit should be stored at the recommended temperature upon receipt. All components in this kit are stable for at least 12 months when stored at the recommended temperature and left unused.

### **Background References:**

- (1) Chen, D.L. et al. (2015) Mol Imaging Biol 17, 384-93.
- (2) Konstantinidou, A.E. et al. (2007) Apoptosis 12, 695-705.
- (3) He, L. et al. (2005) J Immunol Methods 304, 43-59.
- (4) Rayzman, V.M. and Sentry, J.W. (2006) *Hum Antibodies* 15, 71-80.
- (5) Hirata, A. et al. (2004) *J Histochem Cytochem* 52, 1503-9.
  (6) Juan, G. et al. (1998) *Cytometry* 32, 71-7.
- (7) Bernstein, B.E. and Schreiber, S.L. (2002) *Chem Biol* 9,
  - 1167-73.
- (8) Goto, H. et al. (1999) J Biol Chem 274, 25543-9.

The Alexa Fluor<sup>®</sup> dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor<sup>®</sup> dyes (except for Alexa Fluor<sup>®</sup> 430 dye) are covered by pending and issued patents.

Alexa Fluor is a registered trademark of Life Technologies Corporation.

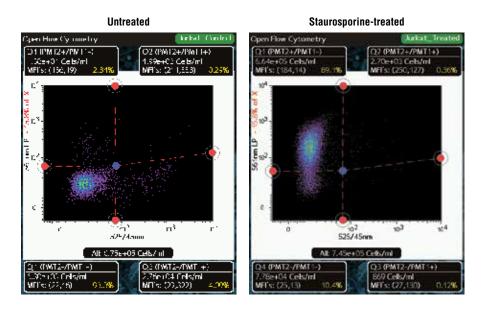
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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse AII—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



CellSimple<sup>™</sup> cell-based analysis of untreated (left panel) and Staurosporine #9953 treated (1  $\mu$ M, 3 hrs; right panel) Jurkat cells using the CellSimple<sup>™</sup> Cleaved Caspase-3 and Phospho-Histone H3 Antibody Assay Kit #75173. Data was collected in both green (525/45 nm) and red channels (561 nm/LP) and analyzed on the Open Flow Cytometry application. Mean fluorescence intensity (MFI) of Cleaved Caspase-3 (Asp175) (D3E9) Rabbit mAb (PE Conjugate) in the red channel (y-axis) and Phospho-Histone H3 (S10) (D2C8) XP<sup>®</sup> Rabbit mAb (Alexa Fluor<sup>®</sup> 488) in the green channel (x-axis) is shown in each dot plot. Cell concentration, MFI, and percentages are displayed in the boxes adjacent to each quadrant. Instrument screen shots are shown.

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# Immunostaining Protocol for CellSimple™ Antibody-based Kits

A. Instrumentation: These kits were specially designed for use with the CellSimple™ Cell Analyzer. However, they may also be used with a flow cytometer or plate reader capable of providing excitation between 480 nm and 490 nm and detecting fluorescent emission between 520 nm and 590 nm.

### B. Kit components:

#75173

- Antibody pair (kit specific)
- 16% Formaldehyde (Methanol-free)
  - NOTE: The screw cap allows for the entire vial contents to be used at once. To extend the product's shelf-life, small volumes should be extracted by piercing the silicone top with a needle and syringe. Store protected from light and use within one month after opening.
- Phosphate Buffered Saline (PBS-20X)

### C. Additional reagents needed, but not supplied.

- 90% methanol
- Bovine Serum Albumin #9998 or equivalent
- Reverse osmosis/deionized (RO/DI) water or equivalent

### D. Reagent preparation

- 1. 1X PBS: To prepare 1 L 1X PBS add 25 ml PBS-20X to 475 ml RO/DI water, mix.
- **2. Incubation Buffer:** Dissolve 0.5 g Bovine Serum Albumin in 100 ml 1X PBS. Store at 4°C.

### E. Fixation

- Collect cells by centrifugation and aspirate supernatant. For adherent cells, remove cells from the plate or flask by trypsinization followed by trypsin neutralization with medium that contains 10% fetal bovine serum.
- 2. Resuspend cells in 0.5 1 ml 1X PBS to 0.5 X  $10^6 5 X 10^6$  cells/ml by gently pipetting up and down. Add 16% Formaldehyde to obtain a final concentration of 4% and vortex gently (for example 335 µl of 16% Formaldehyde added to 1 ml of cell suspension).
- 3. Fix for 10 min at 37°C.
- 4. Chill tubes on ice for 1 min.
- 5. Spin down the cells at 400 x g for 5 min in a chilled microcentrifuge.
- **6.** Carefully remove the supernatant containing formaldehyde into a hazardous waste receptacle.

## F. Permeabilization

- 1. Permeabilize cells by slowly adding 1 1.5 ml of ice-cold 90% methanol. Pipette up and down several times to ensure uniform cell suspension.
- 2. Incubate 30 min on ice.
- **3.** Proceed with immunostaining or store cells at -20°C in 90% methanol.

### G. Immunostaining

- 1. Aliquot 0.5 1 x 10<sup>6</sup> cells into each assay tube.
- 2. Wash the cells twice in 1 ml incubation buffer by centrifugation at 400 x g for 5 min.
- 3. Dilute each of the two fluorochrome-conjugated antibodies 1:50 in incubation buffer. Prepare enough diluted antibody mix to resuspend each of your test samples in 25 µl. For example, for 3 test samples add 1.5 µl of antibody mix to 75 µl of incubation buffer. Note: the antibodies are mixed together to allow a simultaneous detection in both the green and the red channels.
- **4.** Add 25 μl of the diluted antibody mix to each test sample and gently pipette up and down a few times to ensure even cell suspension.
- 5. Incubate for 1 hr at room temperature with occasional gentle mixing.
- 6. Wash once by centrifugation using incubation buffer.
- 7. Resuspend cells in 0.1 0.2 ml PBS and analyze 75 µl on CellSimple™ Cell Analyzer using the Open Flow Cytometry application and using both Green (525/45 nm) and Red (561 nm LP) channels. For more information on how to use the Open Flow Cytometry application and detailed instructions on how to operate the CellSimple™ Cell Analyzer please refer to the CellSimple user guide.

at C	ell Signaling	G Y <sup>®</sup>
SAFETY DATA SHEET (SDS): Ac Issuing Date: 2014-04-02	cording to the OSHA Hazard Communication Standard 29 CFR 1910.1200 Revision Date: 2014-05-07	Version: 1
	SECTION 1. Identification	
Product identifier Product No. Product name UN number Other means of identification	12606 1% Formaldehyde, Methanol-Free UN3334 12606S, 12606P, 12606P2	
Recommended use of the chemi	cal and restrictions on use	
Identified uses Uses advised against	This product is intended for research purposes only. This product is not intended for use in diagnostic procedures or therapeutics. This product is not intended for use in humans or animals.	
Manufacturer, importer, supplier		
Manufacturer address	Cell Signaling Technology, Inc. 3 Trask Lane Denvers MA 0402	

5 anisers, MA 01923 EEL:+1 078 667 2300 FAX:+1 1978 867 2400 www.celleignal.com support@cellsignal.com 978-867-2300 In case of emergency call CHEMTREC 1-800-424-9300 Website Email address Company phone number Emergency telephone number

SECTION 2. Hazard(s) identification

Classification

Acute oral toxicity	Category 4
Acute dermal toxicity	Category 4
Acute inhalation toxicity	Category 3
Skin corrosion/irritation	Category 2
Serious eye damage/eye irritation	Category 2A
Skin sensitization	Category 1
Germ cell mutagenicity	Category 1B
Carcinogenicity	Category 1A
Specific target organ toxicity - single exposure (STOT SE)	Category 3

GHS Label elements, including precautionary statements

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12606 - 16% Formaldehyde, Methanol-Free	
Indication of any immediate medical attention and special treatment needed	

Treat symptomatically.

Advice for emergency responders

General advice Protection of First-aiders

For further assistance, contact your local Poison Control Center. Ensure that medical personnel are aware of the material(s) involved, and take precautions to protect themselves.

to do so

Revision Date: 2014-05-07

### SECTION 5. Fire-fighting measures

Extinguishing media

Suitable Extinguishing Media Use extinguishing measures that are appropriate to local circumstances and the surrounding environment. Unsuitable Extinguishing Media CAUTION: Use of water spray when fighting fire may be inefficient.

Specific hazards arising from the chemical

#### No information available.

Explosion Data

Sensitivity to Mechanical Impact None. Sensitivity to Static Discharge None.

## Protective Equipment and Precautions for Firefighters

As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

#### SECTION 6. Accidental release measures

Personal precautions, protective equipment and emergency procedures

For non-emergency personnel Ensure adequate ventilation. Other information No information available.

#### Environmental precautions

See Section 12 for additional information.

ds and material for containment and cleaning up

methodo and material for conta	innent und eleaning up
Methods for containment	Prevent further leakage or spillage if safe

Methods for cleaning up	Pick up and transfer to properly labeled containers.	
	SECTION 7. Handling and storage	
Descentions for safe bandling		

Precautions for safe handling

Handle in accordance with good industrial hygiene and safety practice

### Conditions for safe storage, including any incompatibilities

Technical measures/Storage conditions	Keep containers tightly closed in a dry, cool and well-ventilated place.
Packaging material	No information available.
Incompatible products	Strong oxidizing agents, Alkalis, Acids, Phenols, Urea.

12606 - 16% Formaldehyde, Methanol-Free



## Signal Word Danger

Hazard statement(s) Harmful if swallowed Harmful in contact with skin Toxic if inhaled Causes skin irritation Causes serious eye irritation May cause an allergic skin reaction May cause genetic defects May cause cancer May cause respiratory irritation

Precautionary Statement(s) Obtain special instructions before use Wear protective gloves/protective clothing/eye protection/face protection Cail a POISON CENTER or doctor/physician if you feel unwell IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing IF INATE: Dr. Remove vicitint for tesh air and keep at rest in a position comfortable for breathing Store in a well-ventilated place. Keep container tightly closed Supplementary Hazard Information

Hazards not otherwise classified (HNOC) None

	SECTION 3. Comp	osition/information on ingre	edients
Formula	CH <sub>2</sub> O		
Chemi	cal Name	CAS No.	Weight %
formaldehyde	(non-stabilised)	50-00-0	10-30
	SECTIO	N 4. First-aid measures	
Eye contact	Rinse thoroughl Consult a physic		minutes, lifting lower and upper eyelids
Skin contact	Wash skin with		
Inhalation	Move to fresh ai	r.	
Indection	Pince mouth		

Most important symptoms and effects, both acute and delayed

Low-dose acute exposure can result in headache, rhinitis, and dyspnea; higher doses may cause severe mucous membrane irritation, burning, and lacrimation, and lower respiratory effects such as bronchitis, pulmonary edema, or pneumonia. Sensitive individuals may experience astimus and dermatilis, even at very low doses. Ocular exposure to formaldehyde vapors produces irritation and lacrimation. Depending on the concentration, formaldehyde solutions may cause transient disconfort and irritation or more severe effects, including comeal opartication and loss of vision. Formaldehyde is astordeet through intact skin and may cause irritation or allergic dermatitis. Ingestion may cause corrosive injury to the gastrointestinal mucosa, with nausea, vomiting, pain, bleeding, and perforation. Systemic effects include metabolic acidosis, CNS depression and coma, respiratory distress, and renal failure.

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### 12606 - 16% Formaldehyde, Methanol-Free

SECTION 8. Exposure controls/personal protection

Control parameters

	Occupational exposu	re limit values	
Chemical Name	ACGIH TLV	OSHA PEL	NIOSH REL
formaldehyde (non-stabilised)	Ceiling: 0.3 ppm	TWA : 0.75 ppm STEL: 2 ppm	IDLH : 20 ppm TWA : 0.016 ppm Ceiling: 0.1 ppm

#### Appropriate engineering controls

Showers, eyewash stations, and ventilation systems.

Individual protection measures, such as personal protective equipment

Personal protective equipment (PPE) needs to be selected depending on the implemented engineering controls, frequency/duration of work activities and the concentrations of the hazardous substance.

res/clothing. e exceeded or irritation is experienced, NIOSH/MSHA approved n should be worn. Positive-pressure supplied air respirators may be some contaminant concentrations. Respiratory protection must be
nce with current local regulations. smoke when using this product. Remove and wash contaminated se.

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Information on basic physical and chemical properties

Physical state	Liquid
Appearance	Clear
Odor	Pungent Characteristic
Color	Light yellow
Odor Threshold	No information available
pH	5.4 @ 20 °C
Melting point/freezing point	No information available
Initial boiling point and boiling	-19.5 °C (formaldehyde)
range	
Flash point	185 °C Closed cup (37% formaldehyde, methanol-free)
Evaporation rate	No information available
Flammability (solid, gas)	No information available
Upper flammability limit	73% No information available.
Lower flammability limit	7% No information available.
Vapor pressure	3,890 mm Hg @ 25 °C (formaldehyde)
Vapor density	No information available
Relative density	No information available
Solubility	No information available.
Solubility in other solvents	No information available
Partition coefficient: n-octanol/wa	
Autoignition temperature	No information available
Decomposition temperature	No information available.
Explosive properties	No information available
Oxidizing properties	No information available
VOC content	No information available
Viscosity	No information available.
Density	No information available.

Revision Date: 2014-05-07

Revision Date: 2014-05-07

#### 12606 - 16% Formaldehyde, Methanol-Free

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Baulaian Date: 2014 05 07

	SECTION 10. Stab	ility and reactivity	
Reactivity			
No information available.			
Chemical stability			
Stable under recommended storage	conditions. Sealed with nitrog	gen gas.	
Possibility of hazardous reactions	1		
Hazardous reactions Hazardous polymerization	None under normal proces Polymerization can occur.	ssing.	
Conditions to Avoid			
No information available.			
Incompatible Materials			
Strong oxidizing agents, Alkalis, Acie	ds, Phenols, Urea.		
Hazardous Decomposition Produc	cts		
None under normal use. Reacts with	HCI to form bis-Chloromethy	l ether.	
None under normal use. Reacts with	HCI to form bis-Chloromethy SECTION 11. Toxico		
	SECTION 11. Toxico		
None under normal use. Reacts with Information on likely routes of exp Inhalation Eye contact Skin contact Ingestion	SECTION 11. Toxico osure Vapours may irritate throat Vapor may cause irritation Irritating to skin. Repeated susceptible persons. Harr	blogical information t and respiratory system. Toxic by . Contact with eyes may cause if or prolonged skin contact may ca	itation. ause allergic reactions with
nformation on likely routes of exp Inhalation Eye contact Skin contact Ingestion	SECTION 11. Toxico Obsure Vapours may irritate throat Vapor may cause irritation Irritating to skin. Repeated susceptible persons. Harr Harrful if swallowed. Inge diarrhea.	blogical information t and respiratory system. Toxic by . Contact with eyes may cause irr or prolonged skin contact may co ful in contact with skin.	itation. ause allergic reactions with
Information on likely routes of exp Inhalation Eye contact Skin contact	SECTION 11. Toxico OSUTE Vapours may irritate throat Vapor may cause irritation Irritating to skin. Repeated susceptible persons. Harr Harrhul if swallowed. Inge diarrhea. Is. b, or under the close super	Logical information and respiratory system. Toxic by Contact with eyes may cause in or prolonged skin contact may ci- ful in contact with skin. stion may cause gastrointestinal I vision of, those property qualified	itation. ause allergic reactions with irritation, nausea, vomiting and in the handling and use of
Information on likely routes of exp Inhalation Eye contact Skin contact Ingestion Information on toxicological effec This material should only be handles Detentially hazerdous chemicals. It	SECTION 11. Toxico OSUTE Vapours may irritate throat Vapor may cause irritation Irritating to skin. Repeated susceptible persons. Harr Harrhul if swallowed. Inge diarrhea. Is. b, or under the close super	Logical information and respiratory system. Toxic by Contact with eyes may cause in or prolonged skin contact may ci- ful in contact with skin. stion may cause gastrointestinal I vision of, those property qualified	itation. ause allergic reactions with irritation, nausea, vomiting and in the handling and use of

formaldehyde (non-stabilised)	= 100 mg/kg (Rat)	= 270 mg/kg (Rabbit)	= 250 ppm ( Rat ) 4 h
NOAEL Oral Value LOAEL Oral Value	Rat 2-Year Bioassay: 15 n Rat 2-Year Bioassay: 82 n	ng/kg/day ng/kg/day (Reduced weight gain,	histopathology in rats)

Delayed and immediate effects as well as chronic effects from short and long-term exposure

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Symptoms Skin corrosion/irritation Serious eye damage/eye irritation	cause severe mucous me effects such as bronchitis experience asthma and di formaldehyde vapors proc formaldehyde volutions m effects, including corneal through intact skin and m corrosive injury to the gas	can result in headache, rhinitis, michane intration, burning, and lau , pulmonary edema, or pneumoni ermatitis, even a turvy lova dosse duces intration and lacrimation. D ay cause transient discomfort and opacification and loss of vision. F ay cause intratistication and loss of vision. F a cla include metabolic acidosis, Cl enal failure.	crimation, and lower respirat a. Sensitive individuals may . Ocular exposure to epending on the concentrati d irritation or more severe ormaldehyde is absorbed natitis. Ingestion may cause a, vomiting, pain, bleeding, a
Corrosivity	No information available.		
Sensitization	May cause sensitization of susceptible persons.		
Mutagenic effects	No specific testing was done on this product. Mutagenic testing of the hazardous ing		
-		d in some positive mutagenic res	
Carcinogenicity	The list below indicates a	ny ingredient listed as a carcinoge	en:
Chemical Name	IARC	NTP	OSHA
ormaldehyde (non-stabilised) 50-00-0	1	Known	x
Legend: IARC: (International Agency for NTP: (National Toxicity Program OSHA: (Occupational Safety & F	) Known - Known Carcinogen	-	
IARC: (International Agency for NTP: (National Toxicity Program	h) Known - Known Carcinogen dealth Administration) X - Presen There is limited evidence	nt that formaldehyde causes advers	
ARC: (International Agency for NTP: (National Toxicity Program OSHA: (Occupational Safety & H Reproductive toxicity	b) Known - Known Carcinogen Health Administration) X - Preser There is limited evidence Formaldehyde has not be human teratogen at occup	nt	
IARC: (International Agency for NTP: (National Toxicity Program OSHA: (Occupational Safety & H Reproductive toxicity STOT - single exposure	a) Known - Known Carcinogen lealth Administration) X - Presen There is limited evidence Formaldehyde has not be human teratogen at occup Respiratory system.	nt that formaldehyde causes advers en proven to be teratogenic in an	
IARC: (International Agency for NTP: (National Toxicity Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure	b) Known - Known Carcinogen dealth Administration) X - Presei There is limited evidence Formaldehyde has not be human teratogen at occup Respiratory system. No information available.	nt that formaldehyde causes advers en proven to be teratogenic in an	
IARC: (International Agency for NTP: (National Toxicity Program OSHA: (Occupational Safety & H Reproductive toxicity STOT - single exposure STOT - repeated exposure STOT - repeated exposure	a) Known - Known Carcinogen Health Administration) X - Preser There is limited evidence Formaldehyde has not be human teratogen at occup Respiratory system. No information available. No information available.	nt that formaldehyde causes advers en proven to be teratogenic in an	
IARC: (International Agency for NTP: (National Toxicity Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure	b) Known - Known Carcinogen lealth Administration) X - Presei There is limited evidence Formaldehyde has not be human teratogen at occup Respiratory system. No information available. No information available.	nt that formaldehyde causes advers en proven to be teratogenic in an pationally permissible levels.	
IARC: (International Agency for NTP: (National Toxicity Program OSHA: (Occupational Safety & H Reproductive toxicity STOT - single exposure STOT - repeated exposure STOT - repeated exposure	b) Known - Known Carcinogen lealth Administration) X - Presei There is limited evidence Formaldehyde has not be human teratogen at occup Respiratory system. No information available. No information available.	nt that formaldehyde causes advers en proven to be teratogenic in an	
IARC: (International Agency for NTP: (National Toxicity Program OSHA: (Occupational Safety & H Reproductive toxicity STOT - single exposure STOT - repeated exposure STOT - repeated exposure	b) Known - Known Carcinogen lealth Administration) X - Presei There is limited evidence Formaldehyde has not be human teratogen at occup Respiratory system. No information available. No information available.	nt that formaldehyde causes advers en proven to be teratogenic in an pationally permissible levels.	
IARC: (International Agency for MTP: (National Toxicly Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure Neurological effects Aspiration Hazard	I) Known Carcinogen dealth Administration) X - Presen tealth Administration X - Presen Thrers is limited evidence Formaldehyde has not be human teratogen at occur Respiratory system. No information available. No information available. SECTION 12. Ecol	nt that formaldehyde causes advers en proven to be teratogenic in an nationally permissible levels.	
IARC: (International Agency for MTP: (National Toxicly Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure Neurological offects Aspiration Hazard	I) Known Carcinogen dealth Administration) X - Presen tealth Administration X - Presen Thrers is limited evidence Formaldehyde has not be human teratogen at occur Respiratory system. No information available. No information available. SECTION 12. Ecol	nt that formaldehyde causes advers en proven to be teratogenic in an nationally permissible levels.	imals and is probably not a
IARC: International Agency for MTP: (National Toxicity Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure Neurological offects Aspiration Hazard <u>toxicity</u> duct does not present an aquat Chemical Name	I) Known Carcinogén Health Administration X - Prese Formaldéhyde has not be human terdogen al occur Respiratory system. No information available. SECTION 12. Ecol ic toxicity hazard based on kno	nt that formaldehyde causes advers en proven to be teratogenic in an actionally permissible levels. logical information own or supplied information. Toxicity to fish	Toxicity to daphnia and ot aquatic invertebrates
IARC: International Agency for MTP: (National Toxicity Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure STOT - repeated exposure Neurological offects Aspiration Hazard Moxicity duct does not present an aquat	I) Known Carcinogén Health Administration X - Prese Formaldéhyde has not be human terdogen al occur Respiratory system. No information available. SECTION 12. Ecol ic toxicity hazard based on kno	nt that formaldehyde causes advers en proven to be teratogenic in an aationally permissible levels. logical information own or supplied information. Toxicity to fish LCS0 41 mgl. (Brachydanio rerio)	Toxicity to daphnia and ot aquatic invertebrates EC50 11.3 - 18 mg/L (Daph
IARC: International Agency for MTP: (National Toxicity Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure Neurological offects Aspiration Hazard <u>toxicity</u> duct does not present an aquat Chemical Name	I) Known Carcinogén Health Administration X - Prese Formaldéhyde has not be human terdogen al occur Respiratory system. No information available. SECTION 12. Ecol ic toxicity hazard based on kno	nt that formaldehyde causes advers en proven to be teratogenic in an ationally permissible levels.  logical information own or supplied information.  Toxicity to fish LCS0 41 mgL (Brazhydanic rerio) 96 h LCS0 42207 mgL (Primephales promals) 96 h LCS0	Toxicity to daphnia and ot aquatic invertebrates EC50 11.3 - 18 mg/L (Daph
IARC: International Agency for MTP: (National Toxicity Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure Neurological offects Aspiration Hazard <u>toxicity</u> duct does not present an aquat Chemical Name	I) Known Carcinogén Health Administration X - Prese Formaldéhyde has not be human terdogen al occur Respiratory system. No information available. SECTION 12. Ecol ic toxicity hazard based on kno	nt that formaldehyde causes advers en proven to be teratogenic in an ationally permissible levels. logical information Toxicity to fish C50 44 mpl. (Brachydanio refo) 96 h L502 22 - 207 mpl. (Pimephales promeias 96 h L503	Toxicity to daphnia and of aquatic invertebrates EC50 11.3 - 18 mpl. (Daph magna) 48 1. LC20 zmgl. (Da
IARC: International Agency for MTP: (National Toxicity Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure Neurological offects Aspiration Hazard <u>toxicity</u> duct does not present an aquat Chemical Name	I) Known Carcinogén Health Administration X - Prese Formaldéhyde has not be human terdogen al occur Respiratory system. No information available. SECTION 12. Ecol ic toxicity hazard based on kno	nt that formaldehyde causes advers en proven to be teratogenic in an ationality permissible levels. logical information over or supplied information. Toxicity to fish LCS0 41 mgL (Brachydanio refo) % th LC30 42.2-2.7 mgL Gif 101 0.21.4 mgL (Brachydanio refo) % th LC30 1.23.7 mgL 0.11.1 mgL (Brachydanio refo) % th LC30 1.23.7 mgL 0.11.1 mgL (Brachydanio refo) % th LC30 1.23.7 mgL 0.11.1 mgL % th LC30 1.23.7 mgL % th LC30 1.23.	Toxicity to daphnia and of aquatic invertebrates EC50 11.3 - 18 mpl. (Daph mgma) 48 h LC20 mpl. (Daph
IARC: International Agency for MTP: (National Toxicity Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure Neurological offects Aspiration Hazard <u>toxicity</u> duct does not present an aquat Chemical Name	I) Known Carcinogén Health Administration X - Prese Formaldéhyde has not be human terdogen al occur Respiratory system. No information available. SECTION 12. Ecol ic toxicity hazard based on kno	nt that formaldehyde causes advers en proven to be teratogenic in an ationally permissible levels. logical information Toxicity to fish C50 44 mpl. (Brachydanio refo) 96 h L502 22 - 207 mpl. (Pimephales promeias 96 h L503	Toxicity to daphnia and of aquatic invertebrates EC50 11.3 - 18 mpl. (Daph mgma) 48 h LC20 mpl. (Daph
IARC: International Agency for MTP: (National Toxicity Program OSHA: (Occupational Safety & F Reproductive toxicity STOT - single exposure STOT - repeated exposure Neurological offects Aspiration Hazard <u>toxicity</u> duct does not present an aquat Chemical Name	I) Known Carcinogén Health Administration X - Prese Formaldéhyde has not be human terdogen al occur Respiratory system. No information available. SECTION 12. Ecol ic toxicity hazard based on kno	nt that formaldehyde causes advers en proven to be teratogenic in an ationally permissible levels.  logical information cover or supplied information. Toxicity to fish LC50 41 mgL (Brachydanio refo) % h LC50 23 - 23 - 70 mgL % h LC50 23 - 20 - 70 mgL % h LC50 20	Toxicity to daphnia and of aquatic invertebrates EC50 11.3 - 18 mg/L Que magna) 48 h Lomg L Que Magna) 48 h

Mobility	ent due to its water solubility
-	· · · · · · · · · · · · · · · · · · ·
Ch	Octanol-Water Partition Coefficient

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Revision Date: 2014-05-07 12606 - 16% Formaldehyde, Methanol-Free

No information available.

### SECTION 13. Disposal considerations

Waste Disposal Methods

Dispose of in accordance with all applicable national environmental laws and regulations.

Disposal considerations

Do not empty into drains; dispose of this material and its container in a safe way.

#### SECTOIN 14. Transport information

This material is subject to regulation as a hazardous material for shipping when offered or intended by aircraft.

### DOT

UN number UN proper shipping name Transport hazard class(es) Special precautions for user Emergency Response Guide Number UN3334 Aviation regulated liquid, n.o.s. (formaldehyde) 9 A35, A189 171

#### IATA

UN3334 Aviation regulated liquid, n.o.s. (formaldehyde) 9 III A27 UN number UN proper shipping name Transport hazard class(es) Packing Group Special precautions for user

#### SECTION 15. Regulatory information North American Inventory Listing

North American Inventory List	ing			
Chemical Name	TSCA 8(b)	TSCA 12(b)	DSL	NDSL
formaldehyde (non-stabilised)	Listed	Not Listed	Listed	Not Listed

#### SARA 313

Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product contains a chemical or chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372:

Chemical Name	CAS No.	SARA 313 - Threshold Values %
formaldehyde (non-stabilised)	50-00-0	0.1
SARA 311/312 Hazard Categories	Yes	
Chronic Health Hazard	Yes	
Fire Hazard	No	
Sudden Release of Pressure Hazard	No	
Reactive Hazard	No	

### Clean Water Act

This product contains the following substances which are regulated pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42):

#### 12606 - 16% Formaldehyde, Methanol-Free

Revision Date: 2014-05-07

Chemical Name	CWA - Reportable Quantities	CWA - Toxic Pollutants	CWA - Priority Pollutants	CWA - Hazardous Substances	CWA - Bioaccumulative Chemicals of Concern (BCCs)
formaldehyde (non-stabilised)	100 lb	Not Listed	Not Listed	Listed	Not Listed

#### CERCLA

This material, as supplied, contains one or more substances regulated as a hazardous substance under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302):

#### Chemical Name Hazardous Substances RQs Extremely Hazardous Substances RQs 100 lb formaldehyde (non-stabilised) 100 lb

California Proposition 65

#### This product contains the following Proposition 65 chemicals

Chemical Name	California Prop. 65
formaldehyde (non-stabilised)	Carcinogen

### U.S. State Right-to-Know Regulations

This product contains the following U.S. State Right to Know chemicals:

Chemical Name	New Jersey	Massachusetts	Pennsylvania
formaldehyde (non-stabilised)	Listed	Listed	Listed

#### U.S. FIFRA Label Information

This product does not contain any substances regulated as pesticides.

US Commerce Department - Export Administration Regulations Information

This product does not contain any substances regulated under the Chemical Weapons Convention (CWC).

U.S. Drug Enforcement Administration Information

This product does not contain any substances regulated under the DEA.

#### SECTION 16. Other information

Issuing Date: 2014-04-02 Revision Date: 2014-05-07

Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of Safety Data Sheet



Safety Data Sheet (SDS) According to the OSHA Hazard Communication Standard 29 CFR 1910.1200

Issuing Date: 2014-02-24	Revision Date: 2014-02-24	Version: 1
	SECTION 1. Identification	
roduct identifier		
Product number	9808	
roduct name	Phosphate Buffered Saline (PBS-20X)	
ther means of identification	9808BC, 9808P, 9808P2, 9808S	
ecommended use of the chemic	al and restrictions on use	
dentified uses	This product is intended for research purpos	es only.
Jses advised against	This product is not intended for use in diagnormal This product is not intended for use in huma	
Anufacturer, importer, supplier	I his product is not intended for use in huma	ns or animais.
analactarol, importor, cappilor		
lanufacturer address	Cell Signaling Technology, Inc.	
	3 Trask Lane Danvers, MA 01923	
	United States	
	TEL: +1 978 867 2300	
	FAX: +1 978 867 2400	
Vebsite	www.cellsignal.com	
mail address	support@cellsignal.com	
mergency telephone number	In case of emergency call CHEMTREC 1-80	0-424-9300
	SECTION 2. Hazard(s) identifica	ation
Classification		
This substance/mixture is considere	d hazardous by the 2012 OSHA Hazard Comm	unication Standard (29 CFR 1910.1200)
Serious eye damage/eye irritation	1	Category 2B
GHS Label elements, including p	ecautionary statements	
Signal Word		
Varning		
Hazard statement(s) Causes eye irritation.		

Precautionary Statement(s) Wash face, hands and any exposed skin thoroughly after handling. IF IN EYES: Rines cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.

Supplementary Hazard Information No information available.

SECTION 3. Composition/information on ingredients

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9808 - Phosphate Buffered Saline (PE	BS-20X) Revision Date: 2014-02-
Methods and material for contain	ment and cleaning up
Methods for containment Methods for cleaning up	Prevent further leakage or spillage if safe to do so. Pick up and transfer to properly labeled containers.
	SECTION 7. Handling and storage
recautions for safe handling	
andle in accordance with good ind	ustrial hygiene and safety practice.
onditions for safe storage, inclu	ding any incompatibilities
Technical measures/Storage conditions	Keep containers tightly closed in a dry, cool and well-ventilated place.
Packaging material	No information available. None known based on information supplied.
SE	CTION 8. Exposure controls/personal protection
ontrol parameters	
nis product, as supplied, does not becific regulatory bodies.	contain any hazardous materials with occupational exposure limits established by the region
ppropriate engineering controls	_
howers, eyewash stations, and ver	ntilation systems.
dividual protection measures, s	such as personal protective equipment
	<ol> <li>needs to be selected depending on the implemented engineering controls, and the concentrations of the hazardous substance.</li> </ol>
Eye/face protection Skin and body protection Respiratory protection Hygiene measures	Safety glasses with side-shields. Wear protective gloves/clothing. If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Positive-pressure supplied air respirators may be required for high airborne contaminant concentrations. Respiratory protection must be provided in accordance with ournert local regulations. Handle in accordance with ourner local regulations.
	SECTION 9. Physical and chemical properties
	1 chemical properties
Physical state Appearance Color	Liquid No information available Colorless
hysical state uppearance olor Ddor	No information available
nformation on basic physical and Physical state uppearance Jolor Jolor Threshold IH	No information available Colorless No information available No information available 7.4
Physical state Appearance Color dor Ddor Threshold	No information available Colorless No information available No information available
hysical state ppearance bolor door Threshold H Melting point/freezing point nitial boiling point and boiling ange	No information available Coloriess No information available No information available 7.4 No information available No information available
hysical state ppearance bolor Ddor Threshold Helting point/freezing point hital boiling point and boiling ange Tash point	No information available Colorless No information available No information available 7.4 No information available
hysical state ppearance olor dor Threshold H elting point/freezing point litial boiling point and boiling nge	No information available Colorless No information available No information available 7.4 No information available No information available

9808 - Phosphate Buffered Saline (PBS-20X)

Chemical hature	Aqueous but	fer solution		
Chemical Nam	e	CAS No	Weight %	
sodium chlorid	e	7647-14-5	10-30	

Revision Date: 2014-02-24

	SECTION 4. First-aid measures			
Eye contact	Rinse thoroughly with plenty of water for at least 15 minutes, lifting lower and upper eyelic Consult a physician.			
Skin contact	Wash skin with soap and water. Move to fresh air.			
Ingestion	If swallowed, do not induce vomiting - seek medical advice.			
Most important symptoms and effect	cts, both acute and delayed			
No information available.				
Indication of any immediate medica	l attention and special treatment needed			
Treat symptomatically.				
Advice for emergency responders				
General advice Protection of first-aiders	For further assistance, contact your local Poison Control Center. Ensure that medical personnel are aware of the material(s) involved, and take precautions to protect themselves.			
	SECTION 5. Fire-fighting measures			
Extinguishing media				
Suitable Extinguishing Media	Use extinguishing measures that are appropriate to local circumstances and the			
Unsuitable Extinguishing Media	surrounding environment. CAUTION: Use of water spray when fighting fire may be inefficient.			
Specific hazards arising from the ch	nemical			
No information available.				
Explosion Data				
Sensitivity to Mechanical Impact Sensitivity to Static Discharge				
Protective Equipment and Precaution	ons for Firefighters			

ed breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) a As in any fire, w protective gear.

SECTION 6. Accidental release measures

Personal precautions, protective equipment and emergency procedures\_

For non-emergency personnel Ensure adequate ventilation. No information available.

Environmental precautions

See Section 12 for additional information.

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9808 - Phosphate Buffered Saline (P	BS-20X)	Revision Date: 2014-02-2
Lower flammability limit	No information available.	
Vapor pressure	No information available	
Vapor density	No information available	
Relative density	No information available	
Solubility	No information available.	
Solubility in other solvents	No information available	
Partition coefficient: n-octanol/w Autoignition temperature	No information available	
Decomposition temperature	No information available	
Explosive properties	No information available	
Oxidizing properties	No information available	
VOC content	No information available	
Viscosity	No information available.	
Density	No information available.	
Solubility in other solvents	No information available	
	SECTION 10. Stability and reactivity	
Reactivity		
No information available.		
Chemical stability		
Stable under recommended storag	e conditions.	
Possibility of hazardous reaction	<u>s</u>	
Hazardous reactions Hazardous polymerization	None under normal processing. None under normal processing.	
Conditions to Avoid		
No information available.		
Incompatible Materials		
None known based on information	supplied.	
Hazardous Decomposition Produ	icts_	
None known based on information	supplied.	
	SECTION 11. Toxicological information	
Information on likely routes of ex	posure	
Inhalation	There is no data available for this product.	
Eye contact	May cause temporary eye irritation.	
Skin contact	There is no data available for this product.	
Ingestion	There is no data available for this product.	
Information on toxicological effe	cts	
	d by, or under the close supervision of, those properly qua should be borne in mind that the toxocological and physio	

not well defined.

Chemical Name	LD50 Oral	LD50 Dermal	LC50 Inhalation
sodium chloride	3000 mg/kg ( Rat )	10 g/kg (Rabbit)	42 g/m <sup>3</sup> (Rat) 1 h

Symptoms	No information available.
Sensitization	No information available.
Mutagenic effects	No information available.
Carcinogenicity	No component of this product present at levels greater than or equal to 0.1% is identifial as probable, possible or confirmed carcinogen by IARC, ACGIH, NTP, or OSHA.
Reproductive toxicity	No information available.
STOT - single exposure	No information available.
STOT - repeated exposure	No information available.
Neurological effects	No information available.
Aspiration Hazard	No information available.

SECTION 12. Ecological information

Chemical Name	Toxicity to algae	Toxicity to fish	Toxicity to daphnia and other aquatic invertebrates
sodium chloride	-	LC50 5560 - 6080 mg/L (Lepomis marcachinus) 96 h LC50 12946 mg/L (Lepomis marcachinus) 96 h LC50 5474 - 7824 mg/L (Oncorhynchus mykiss) 96 h LC50 70550 mg/L (Pimephales promelas) 96 h LC50 6020 - 7070 mg/L (Pimephales promelas) 96 h	EC50 340.7 - 469.2 mg/L (Daphnia magna) 48 h EC50 1000 mg/L (Daphnia magna) 48 h

Persistence and degradability Bioaccumulation Mobility No information available. No information available. No information available

### Other adverse effects

No information available

#### SECTION 13. Disposal considerations

Waste Disposal Methods

Dispose of in accordance with all applicable national environmental laws and regulations.

#### Disposal considerations

Do not empty into drains; dispose of this material and its container in a safe way.

SECTION 14. Transport information Γ

This material is not subject to regulation as a hazardous material for shipping.

SECTION 15. Regulatory information					
North American Inventory Listing					
Chemical Name	TSCA 8(b)	TSCA 12(b)	DSL	NDSL	

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9808 - Phosphate Buffered Saline (PBS-20X)

Revision Date: 2014-02-24

Revision Date: 2014-02-24

Issuing Date: 2014-02-24 Revision Date: 2014-02-24 Disclaimer

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SECTION 16. Other information

End of Safety Data Sheet

9808 - Phosphate Buffered Saline (PBS-20X)

Т

sodium chloride

Listed Canadian Workplace Hazardous Materials Information System (WHMIS) Classification

$\overline{\mathbf{T}}$	Class D2B - Toxic Material at >= 1%

Not Listed

Listed

#### SARA 313

Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372.

Yes No No No No

#### SARA 311/312 Hazard Categories

Acute Health Hazard
Chronic Health Hazard
Fire Hazard
Sudden Release of Pressure Hazard
Reactive Hazard

### Clean Water Act

This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42).

#### CERCLA

This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material.

#### California Proposition 65

This product does not contain any Proposition 65 chemicals.

### U.S. State Right-to-Know Regulations

This product contains the following U.S. State Right to Know chemicals:

Chemical Name	New Jersey	Massachusetts	Pennsylvania
disodium	Listed	Listed	Listed
hydrogenorthophosphate			

U.S. FIFRA Label Information

This product does not contain any substances regulated as pesticides.

US Commerce Department - Export Administration Regulations Information

This product does not contain any substances regulated under the Chemical Weapons Convention (CWC).

#### U.S. Drug Enforcement Administration Information

This product does not contain any substances regulated under the DEA.

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