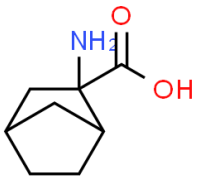
ChemStress® Chemical Functions

Valitacell® Ltd

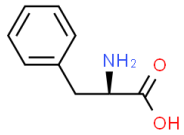
BCH

Chemical Identity		
2-aminobicyclo-(2,2,1)heptane-carboxylic acid		
	SMILES	C1CC2CC1CC2(C(=O)O)N
	InChI	InChI=1S/C8H13NO2/c9-8(7(10)11)4-5-1-2-6(8)3-5/h5-6H,1-4,9H2,(H,10,11)
	ChemSpider	103149

Function	
Function class	Nutrient uptake inhibitor
Primary Target	System L amino acid transporter
Mechanism of Action	A leucine analogue that inhibits System L transport of neutral amino acids. A depleted cytosolic pool of neutral amino acids has been linked to reduced growth in early culture, and to lower mTOR activity leading to diminished protein synthesis.
References	(Nicklin et al., 2009) (Edros et al., 2014)
ChemStress® Rationale	**Cells that struggle to transport neutral amino acids are likely to exhibit low rates of protein synthesis and poor growth.**

Plate location (well)	
position	G11-12

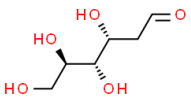
D-phenylalanine -(D-Phe)

Chemical Identity		
D-(+)-Phenylalanine		
	SMILES	c1ccc(cc1)C[C@H](C(=O)O)N
	InChI	InChI=1S/C9H11NO2/c10-8(9(11)12)6-7-4-2-1-3-5-7/h1-5,8H,6,10H2,(H,11,12)/t8-/m1/s1
	ChemSpider	64639

Function	
Function class	Nutrient uptake inhibitor
Primary Target	Inhibits system A amino acid transport
Mechanism of Action	Inhibits essential amino acid uptake in cell culture. L-glutamine and L-leucine transport can be impacted. These amino acids are linked to activing mTORC1, which regulates cell growth according to nutrient status.
References	(Nicklin et al., 2009) (Jewell et al., 2015)
ChemStress® Rationale	**Cells that struggle to uptake amino acids and activate key growth pathways are likely to exhibit altered protein synthesis and poor growth.**

Plate location (well)	
position	A7-9 (1)
position	C4-5 (3)

2-deoxyglucose (2dg)

Chemical Identity		
2-Deoxy-D-glucose		
	SMILES	C(C=O)[C@H]([C@@H]([C@@H](CO)O)O)O
	InChI	InChI=1S/C6H12O5/c7-2-1-4(9)6(11)5(10)3-8/h2,4-6,8-11H,1,3H2/t4-,5-,6+/m1/s1
	ChemSpider	97292

Function	
Function class	Nutrient uptake inhibitor
Primary Target	Competitively inhibits glucose metabolism
Mechanism of Action	An analogue of glucose which can be taken up by cells but not fully metabolised. An intermediate of the chemical accumulates in cells and interferes with carbohydrate metabolism by inhibiting glycolytic enzymes. It can induce growth inhibition and alter glycosylation.
References	(Simons et al., 2007) (Ralser et al., 2008) (Gray et al., 2005)
ChemStress® Rationale	Cells with inhibited glucose metabolism will likely struggle to grow. The quality of product produced by cells may also be impacted.

Plate Information (well)	
position	E4-6

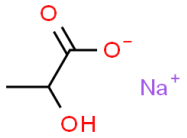
Ammonium chloride (Am.Chl)

Chemical Identity		
Ammonium chloride		
H_4N^+ Cl^-	SMILES	[NH4+].[Cl-]
	InChI	InChI=1S/ClH.H3N/h1H;1H3
	ChemSpider	23807

Function	
Function class	Toxic compound (waste product)
Primary Target	Glutamine pathway inhibition
Mechanism of Action	A common toxic compound that builds up in cell culture as a result of cell metabolism and also decomposition of glutamine in the culture media. High levels of ammonia in culture can cause decreased cell growth, decreased productivity and altered product glycosylation.
References	(Yang and Butler, 2000) (Lao and Toth, 1997)
ChemStress® Rationale	**Cells growing in the presence of waste products will likely struggle to grow and produce product. The quality of the product may also be affected.**

Plate Information (well)	
position	A10-12 (1)
position	B4-6 (2)

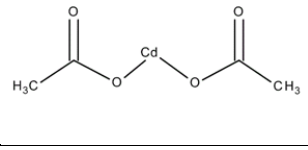
Sodium lactate (NaLac)

Chemical Identity		
(±)-2-Hydroxypropionic acid sodium salt		
	SMILES	CC(C(=O)[O-])O.[Na+]
	InChI	InChI=1S/C3H6O3.Na/c1-2(4)3(5)6;/h2,4H,1H3,(H,5,6);/q;+1/p-1
	ChemSpider	6049

Function	
Function class	Osmolarity modulation
Primary Target	Increase osmolarity
Mechanism of Action	Increased lactate levels are common as cell culture time increases. Lactate reduces growth rate and increases the osmolarity, with cells entering into a stationary phase. The high osmolarity is potentially the cause of reduced growth and cell cycle arrest observed in cells exposed to sodium lactate.
References	(Yoon et al., 2001) (Buchsteiner et al., 2018) (Pan et al., 2017) (Martínez et al., 2013) (Freund and Croughan, 2018)
ChemStress® Rationale	**Cells growing in the presence of waste products and increased osmolarity will likely struggle to grow and produce product.**

Plate Information (well)	
position	H4-6

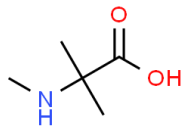
Cadmium acetate dihydrate (Cadm)

Chemical Identity		
Cadmium acetate-2-hydrate		
	SMILES	CC(=O)[O-].CC(=O)[O-].O.O.[Cd+2]
	InChI	InChI=1S/2C2H4O2.Cd.2H2O/c2*1-2(3)4;;;/h2*1H3,(H,3,4);;2*1H2/q;;+2;;/p-2
	ChemSpider	5020634

Function	
Function class	Cell cycle inhibition
Primary Target	Cell cycle arrest
Mechanism of Action	Delays cell cycle progression regardless of cell cycle phase. The arrest of cells in culture can have specific affects e.g. elevated levels of G2/M phase is usually indicative of apoptosis while sustained arrest at G0/G1 phase is linked to differentiation, peak biogenesis and protein expression.
References	(Yang et al., 2004) (Du et al., 2015)
ChemStress® Rationale	**Cells affected may have delays in cell cycle progression and struggle to increase biomass, maintain viability and produce product.**

Plate Information (well)	
position	B7-9 (2)
position	D10-12 (3)

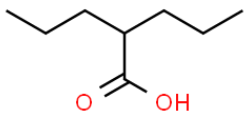
α-(methylamino)isobutyric acid (MeAIB)

Chemical Identity		
2-(Methylamino)-2-methylpropionic acid		
	SMILES	CC(C)(C(=O)O)NC
	InChI	InChI=1S/C5H11NO2/c1-5(2,6-3)4(7)8/h6H,1-3H3,(H,7,8)
	ChemSpider	68242

Function	
Function class	Nutrient deprivation
Primary Target	Inhibits proline transport and system A amino acid transport
Mechanism of Action	An amino acid analogue for system A mediated transport of amino acids into cells. Acts to inhibit system A transport activity decreasing the amino acids in cells.
References	(Curriden and Englesberg, 1981) (Hatanaka et al., 2006) (Hatanaka et al., 2001) (Freeman et al., 1999)
ChemStress® Rationale	Cells that stuggle to uptake amino acids may struggle to produce enough nutrients to grow and manufacture product.

Plate Information (well)	
position	C10-12 (1)
position	G4-6 (2)

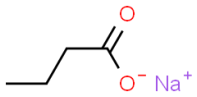
Valproic acid (Val)

Chemical Identity		
2-Propylpentanoic acid		
	SMILES	CCCC(CCC)C(=O)O
	InChI	InChI=1S/C8H16O2/c1-3-5-7(6-4-2)8(9)10/h7H,3-6H2,1-2H3,(H,9,10)
	ChemSpider	3009

Function	
Function class	Histone Deacetylase Inhibitor (HDACi)
Primary Target	Cell cycle inhibition
Mechanism of Action	Shown to block cell growth in culture and increase recombinant protein production in transiently and stably infected cell cultures. Cells exposed to high levels can exhibit increased endoplasmic reticulum (ER) stress and decreased growth and productivity. Can modulate recombinant protein production
References	(Backliwal et al., 2008) (Segar et al., 2017) (Wulhfard et al., 2010) (Yang et al., 2014)
ChemStress® Rationale	**Cells which are sensitive to cell cycle inhibition, are likely to exhibit decreased viability and may exhibit saturation of productivity.**

Plate Information (well)	
position	F4-6

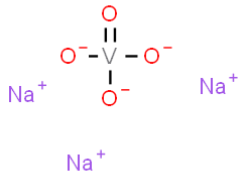
Sodium Butyrate (NaBu)

Chemical Identity		
Butyric acid sodium salt		
	SMILES	CCCC(=O)[O-].[Na+]
	InChI	InChI=1S/C4H8O2.Na/c1-2-3-4(5)6;/h2-3H2,1H3,(H,5,6);/q;+1/p-1
	ChemSpider	8727

Function	
Function class	Histone Deacetylase Inhibitor (HDACi)
Primary Target	Cell cycle inhibition
Mechanism of Action	Can increase cell productivity when cells are exposed to appropriate concentrations. Cells sensitive to high concentrations can exhibit deteriorated product quality, resulting in increased heterogeneity of product glycosylation and decreased cell viability in culture. Can control cell proliferation.
References	(Sung et al., 2004) (Rahimi-Zarchi et al., 2018) (Allen et al., 2008) (Chen et al., 2011) (Avello et al., 2017)
ChemStress® Rationale	Cells which are sensitive to high levels likely exhibit decreased protein production, or deterioration of product quality. Cells will largely exhibit decreased viability in the presence of the chemical.

Plate Information (well)	
position	F1-3

Sodium Orthovanadate (NaOthv)

Chemical Identity		
Sodium vanadium oxide		
	SMILES	[O-][V](=O)([O-])[O-].[Na+].[Na+].[Na+]
	InChI	InChI=1S/3Na.4O.V/q3*+1;;3*-1;
	ChemSpider	55575

Function	
Function class	Cell cycle inhibition
Primary Target	Apoptosis inhibitor
Mechanism of Action	Inhibits protein tyrosine phosphatases (PTP), which modulate proliferation, migration and apoptosis of cells. Sensitive cells may exhibit loss of viability in the presence of the inhibitor. Differential response can be due to genetic background of cells.
References	(Le et al., 2017) (Ruddraraju and Zhang, 2017) (Huyer et al., 1997) (Través et al., 2014) (Khalil and Jameson, 2017)
ChemStress® Rationale	**Cells which are sensitive to apoptosis inhibition will likely exhibit no impact on viability, relative to untreated cells.**

Plate Information (well)	
position	F7-9

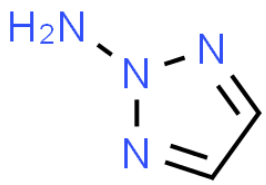
Sodium Chloride (NaCl)

Chemical Identity		
Sodium Monochloride		
$Cl^- \ Na^+$	SMILES	[Na+].[Cl-]
	InChI	InChI=1S/ClH.Na/h1H;/q;+1/p-1
	ChemSpider	5044

Function	
Function class	Osmolarity modulation
Primary Target	Increase osmolarity
Mechanism of Action	Increases the osmolarity of the cell culture environment. Increased secretion of protein may be observed coupled with decreased cell growth. Mimics the hyperosmolarity seen in batch and fed-batch culture
References	(Shen et al., 2010) (Buchsteiner et al., 2018) (Wuest et al., 2012)
ChemStress® Rationale	**Cells which are sensitive to increased osmolarity may typically exhibit decreased viability in these osmotic environments.**

Plate Information (well)	
position	D7-9

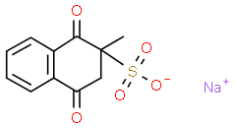
Aminotriazole (AMT)

Chemical Identity		
2-Aminotriazole		
	SMILES	c1cnn(n1)N
	InChI	InChI=1S/C2H4N4/c3-6-4-1-2-5-6/h1-2H,3H2
	ChemSpider	11488551

Function	
Function class	Oxidative stress inducer
Primary Target	Inhibitor of catalase
Mechanism of Action	Decreases the activity of peroxisome catalase, increasing the levels of reactive oxygen species (ROS) (e.g. hydrogen peroxide) produced by cells. Dysregulation of peroxisome catalase is also linked to age-lated damage of cells.
References	(Spitz et al., 1992) (Walton, 2012)
ChemStress® Rationale	Cells will typically exhibit decreases in viability and protein production, lack of sensitivity may indicated robustness against ROS.

Plate Information (well)	
position	C8-9

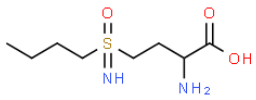
Menadione Sodium Bisulfite (MSB)

Chemical Identity		
2-Methyl-1,4-naphthoquinone sodium bisulfite		
	SMILES	CC1(CC(=O)c2ccccc2C1=O)S(=O)(=O)[O-].[Na+]
	InChI	InChI=1S/C11H10O5S.Na/c1-11(17(14,15)16)6-9(12)7-4-2-3-5-8(7)10(11)13;/h2-5H,6H2,1H3,(H,14,15,16);/q;+1/p-1
	ChemSpider	8219

Function	
Function class	Oxidative stress inducer
Primary Target	Cytotoxin that targets mitochondria
Mechanism of Action	Model compound to promote oxidative stress; disrupts the antioxidant glutathione (GSH). Dysruption of GSH leads to increased sensitivity of cells to oxidants.
References	(Vallis et al., 1997) (Astakhova et al., 2018)
ChemStress® Rationale	Cells sensitive to oxidative stress will likely exhibit decreased viability, product production may not be affected.

Plate Information (well)	
position	B1-3 (4)
position	E7-9 (5)
position	H7-9 (3)

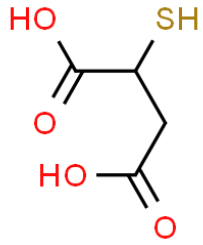
Buthionine Sulfoximine (BSO)

Chemical Identity		
2-Amino-4-(S-butylsulfonimidoyl)butanoic acid		
	SMILES	CCCCS(=N)(=O)CCC(C(=O)O)N
	InChI	InChI=1S/C8H18N2O3S/c1-2-3-5-14(10,13)6-4-7(9)8(11)12/h7,10H,2-6,9H2,1H3,(H,11,12)
	ChemSpider	19896

Function	
Function class	Oxidative stress inducer
Primary Target	Inhibits glutathione (GSH) synthesis
Mechanism of Action	Induces oxidative stress in cells by inhibiting the production of antioxidant GSH. Can increase apoptosis in some cells.
References	(Clark et al., 1984) (Feary et al., 2017) (Tagde et al., 2014)
ChemStress® Rationale	**Cells sensitive to oxidative stress will typically have decreased viability and productivity.**

Plate Information (well)	
position	D1-3

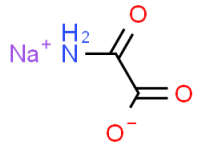
Mercaptosuccinic Acid (MS)

Chemical Identity		
Thiomalic acid		
	SMILES	C(C(C(=O)O)S)C(=O)O
	InChI	InChI=1S/C4H6O4S/c5-3(6)1-2(9)4(7)8/h2,9H,1H2,(H,5,6)(H,7,8)
	ChemSpider	6032

Function	
Function class	Oxidative stress inducer
Primary Target	Inhibits glutathione peroxidases
Mechanism of Action	Inhibits the antioxidative enzyme glutathione peroxidase. Increases the levels of reactive oxygen species (ROS) in cells and can increase the apoptosis of cells.
References	(Quispe et al., 2019) (Ishihara et al., 2011)
ChemStress® Rationale	**Cells which are sensitive to oxidative stress exhibit decrease viability and productivity.**

Plate Information (well)	
position	D4-6

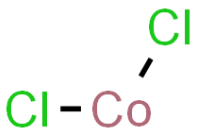
Sodium Oxamate (NaOxam)

Chemical Identity		
Oxalic acid monoamide sodium salt		
	SMILES	C(=O)(C(=O)[O-])N.[Na+]
	InChI	InChI=1S/C2H3NO3.Na/c3-1(4)2(5)6;/h(H2,3,4)(H,5,6);/q;+1/p-1
	ChemSpider	5052

Function	
Function class	Metabolism inhibitor
Primary Target	Inhibits lactate dehydrogenase (LDH)
Mechanism of Action	Targets energy metabolism of cells, interrupting aerobic glycolysis by inhibiting LDH. Reduces the lactate level in cells, which is relevant during fed-batch culture where accumulation of lactate can be high. However, increased levels of respiration have been shown to increase cells sensitivity to oxidative stress.
References	(Zhao et al., 2015) (Kim and Lee, 2007) (Jeong et al., 2006)
ChemStress® Rationale	**Cells which are sensitive can have decreased viability and productivity potentially as a result of increased sensitivity to oxidative stress.**

Plate Information (well)	
position	F1-3

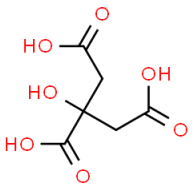
Cobalt chloride (CoCl)

Chemical Identity		
Cobalt(2+) dichloride		
	SMILES	Cl[Co]Cl
	InChI	InChI=1S/2ClH.Co/h2*1H;/q;;+2/p-2
	ChemSpider	22708

Function	
Function class	Hypoxia inducer
Primary Target	Induces hypoxia-inducible factor 1 (HIF-1)
Mechanism of Action	Used to induce hypoxia, by stabilising hypoxia-inducibale factors in cell culture under normoxic conditions.
References	(Wu and Yotnda, 2011) (Gao et al., 2016) (Muñoz-Sánchez and Chánez-Cárdenas, 2019)
ChemStress® Rationale	**Cells which are sensitive generally exhibit decreased viability and productivity in response to hypoxia induction.**

Plate Information (well)	
position	B10-12 (1)
position	H10-11 (2)

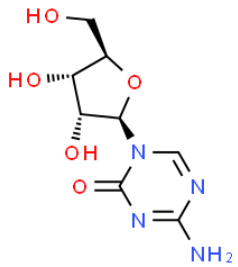
Citric acid (Citric)

Chemical Identity		
b-Hydroxytricarballylic Acid		
	SMILES	C(C(=O)O)C(CC(=O)O)(C(=O)O)O
	InChI	InChI=1S/C6H8O7/c7-3(8)1-6(13,5(11)12)2-4(9)10/h13H,1-2H2,(H,7,8)(H,9,10)(H,11,12)
	ChemSpider	305

Function	
Function class	Modulates pH of culture
Primary Target	Decreases the pH of culture
Mechanism of Action	Reduces the pH of the cell culture environment.
References	(Guimarães et al., 2010)
ChemStress® Rationale	Cells sensitive to decreased pH will typically exhibit decreased proliferation and may have altered cell morphology and product quality.

Plate Information (well)	
position	F10-12

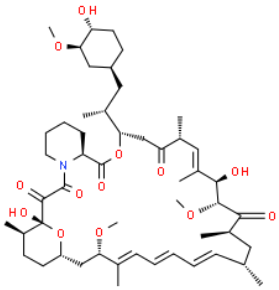
Azacytidine

Chemical Identity		
4-Amino-1-(β-D-ribofuranosyl)-1,3,5-triazin-2(1H)-one		
	SMILES	c1nc(nc(=O)n1[C@H]2[C@@H]([C@@H]([C@H](O2)CO)O)O)N
	InChI	InChI=1S/C8H12N4O5/c9-7-10-2-12(8(16)11-7)6-5(15)4(14)3(1-13)17-6/h2-6,13-15H,1H2,(H2,9,11,16)/t3-,4-,5-,6-/m1/s1
	ChemSpider	9072

Function	
Function class	DNA hypomethylating agent
Primary Target	Methyltransferase inhibitor
Mechanism of Action	An epigenetic modulator which reduces the methylation of DNA. May also impact transgene expression by restoring lost expression.
References	(Takahashi-Hyodo et al., 1999) (Piñero et al., 1999) (Jia et al., 2018)
ChemStress® Rationale	Cells which are sensitive may exhibit increased productivity

Plate Information (well)	
position	A4-6 (2)
position	E10-12

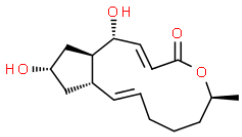
Rapamycin

Chemical Identity		
Sirolimus		
	SMILES	C[C@@H]1CC[C@H]2C[C@@H](/C(=C/C=C/C=C/[C@H](C[C@H](C(=O)[C@@H]([C@@H](/C(=C/[C@H](C(=O)C[C@H](OC(=O)[C@@H]3CCCCN3C(=O)C(=O)[C@@]1(O2)O)[C@H](C)C[C@@H]4CC[C@H]([C@@H](C4)OC)O)C)/C)O)OC)C)C)/C)OC
	InChI	InChI=1S/C51H79NO13/c1-30-16-12-11-13-17-31(2)42(61-8)28-38-21-19-36(7)51(60,65-38)48(57)49(58)52-23-15-14-18-39(52)50(59)64-43(33(4)26-37-20-22-40(53)44(27-37)62-9)29-41(54)32(3)25-35(6)46(56)47(63-10)45(55)34(5)24-30/h11-13,16-17,25,30,32-34,36-40,42-44,46-47,53,56,60H,14-15,18-24,26-29H2,1-10H3/b13-11+,16-12+,31-17+,35-25+/t30-,32-,33-,34-,36-,37+,38+,39+,40-,42+,43+,44-,46-,47+,51-/m1/s1
	ChemSpider	10482078

Function	
Function class	Nutrient deprivation
Primary Target	Inhibitor of mammalian Target of Rapamycin (TOR)
Mechanism of Action	Induces autophagy, a pathway which causes controlled breakdown of cellular compartments, or removal of damaged organelles, generating the components required by the cell for survival during nutrient starvation.
References	(Krampe and Al-Rubeai, 2010) (Codogno and Meijer, 2005)
ChemStress® Rationale	**Cells which are sensitive to nutrient starvation will typically struggle to maintain viability and produce product.**

Plate Information (well)	
position	C1-3 (2)
position	H1-3

Brefeldin A

Chemical Identity		
g,4-Dihydroxy-2-(6-hydroxy-1-heptenyl)-4-cyclopentanecrotonic Acid l-Lactone		
	SMILES	C[C@H]1CCC/C=C/[C@@H]2C[C@@H](C[C@H]2[C@@H](/C=C/C(=O)O1)O)O
	InChI	InChI=1S/C16H24O4/c1-11-5-3-2-4-6-12-9-13(17)10-14(12)15(18)7-8-16(19)20-11/h4,6-8,11-15,17-18H,2-3,5,9-10H2,1H3/b6-4+,8-7+/t11-,12+,13-,14+,15+/m0/s1
	ChemSpider	4449949

Function	
Function class	Protein transport inhibitor
Primary Target	Guanine nucleotide exchange factor (GEF)
Mechanism of Action	Induces endoplasmic reticulum (ER) stress, and reduces protein trafficking between the ER and Golgi apparatus. Processing of glycans may also be affected. May induce apoptosis through ER-stress.
References	(Moon et al., 2012) (Kano et al., 2000) (Yan et al., 1994) (Iurlaro and Muñoz-Pinedo, 2016) (Rao et al., 2004)
ChemStress® Rationale	**Cells which are sensitive to ER-stress may struggle to maintain product quality and viability.**

Plate Information (well)	
position	A1-3 (3)
position	C6-7 (5)
position	G1-3 (4)