A	troubleshooting, 256	cell lysis and staining, 332
Accidental cell death, trauma, 1-2	overview, 23–27	flow cytometry, 333
Acridine orange	riptosome analysis by caspase-8	materials, 330–331
Caenorhabditis elegans apoptosis studies,	immunoprecipitation	overview, 330
414–415, 423	applications, 278–279	recipes, 333–334
Drosophila apoptosis assay	immunoprecipitation, 275–276	tumor tissue collection and cell
applications, 346	materials, 272–273	suspension preparation, 332
embryo staining, 345	overview, 272	mitochondrial transmembrane potential
larva tissue staining, 345–346	protein sample preparation, 273–274	assay. See Mitochondrial
materials, 344–345	recipes, 279	transmembrane potential assay
overview, 344	troubleshooting, 278	overview, 2
recipes, 347	western blot, 276	TUNEL assay. See TUNEL assay
troubleshooting, 346	work flow, 274	Apoptosome
Actinomycin D, apoptosis induction assay	strategies, 250–252	activation in vitro, 183
in hematopoietic cells	DNA content assay by cell cycle stage.	assembly and analysis in vitro
incubation conditions, 31	See Propidium iodide	applications, 104–105
materials, 30–32	Drosophila studies. See Drosophila apoptosis	cell lysate preparation, 100
substitute agents for apoptosis induction,	extrinsic, 2–3	dATP activation of lysates, 100-102
31–32	Hoechst 33342 nuclear staining. See Hoechst	fluorimetric analysis of caspase activity,
Annexin V and propidium iodide uptake assay	33342	102-103
applications, 78, 260, 263	inducers, 21–22	gel filtration, 102
controls, 260	intrinsic, 2	materials, 98–99
distinguishing apoptosis from necrosis, 75, 258	mammalian in vivo measurements	overview, 98, 101
flow chart, 76	immunohistochemistry	procaspase preparation for bioassay,
flow cytometry, 76–77	B-cell lymphoma transplantation in	103-104
fluorescence-activated cell sorting, 260–262	mice and apoptosis induction,	recipes, 105–106
materials, 75, 258–259	325-326	SDS-PAGE and western blot, 103
recipes, 79	fragmented DNA detection, 327–328	troubleshooting, 104
staining, 76, 259	materials, 324–325	overview, 95–96
time-lapse imaging	overview, 324	Apoptotic protease activating factor-1 (APAF-1),
applications, 270–271	recipes, 328–329	142, 181
cell preparation, 265, 267–268	troubleshooting, 328	Autophagy-dependent cell death
data analysis, 269	tumor tissue collection and fixation,	assays. See Caenorhabditis elegans autophagy;
materials, 264-265	326–327	Drosophila autophagy;
microscopy, 266-269	overview, 313–315	Mammalian cell autophagy
overview, 264	positron emission tomography of	distinguishing from other cell death
troubleshooting, 269-270	hematological tumor response	modalities, 25
troubleshooting, 77–78	to drugs	inhibitors and inducers, 281
APAF-1. See Apoptotic protease activating	applications, 318	LC3. See MAP1LC3B
factor-1	B-cell lymphoma transplantation in	overview, 5, 279–280
APO-1/Fas. See Death-inducing signaling complex	mice, 317	types, 279
Apoptosis	data analysis, 318	
actinomycin D induction. See Actinomycin D	fluorodeoxyglucose administration	В
Bcl-2 proteins. See Bak; Bax	and uptake measurement,	Bak
Caenorhabditis studies. See Caenorhabditis	317–318	activation assay overview, 116
elegans apoptosis	materials, 316–317	blue native PAGE and antibody gel shift
caspase-activating complexes. See	overview, 316	assay of conformational change
Apoptosome; Death-inducing	recipes, 319	and oligomerization
signaling complex	positron emission tomography of solid	applications, 138
caspase cleavage. See Caspases	tumor response to drugs	cell permeabilization, 136
cytochrome c release assay. See Cytochrome	applications, 323	gel electrophoresis, 137
c release assay	data analysis, 323	materials, 135-136
distinguishing from other cell death	fluorodeoxyglucose administration	overview, 135
modalities. See also Annexin V and	and uptake measurement, 322	recipes, 138-139
propidium iodide uptake assay	materials, 320-321	troubleshooting, 138
cell lines for study, 252	overview, 320	conformational change in activation
crystal violet viability assay	tumor cell transplantation in mice,	flow cytometry
cell preparation, 255	321-322	applications, 129
materials, 254	propidium iodide staining	cell harvesting, 128
overview, 254	B-cell lymphoma transplantation in	conformation-specific antibodies, 128
recipes, 256	mice and apoptosis induction,	flow cytometry, 128
etaining and measurement 256	331_332	materials 127_128

Bak (Continued)	recipes, 120-121	materials, 428-429
overview, 127	troubleshooting, 120	overview, 428
recipes, 129	western blot, 120	recipes, 54
troubleshooting, 128-129	Bcl-2 proteins. See Bak; Bax	troubleshooting, 432
immunoprecipitation analysis	β-N-Acetyl glucosaminidase (NAG)	light microscopy visualization
cell harvesting, 123	cytosolic cathepsin and β-N-acetyl	applications, 411–412
cell lysis, 124	glucosaminidase activity assays	data analysis, 412
detergents, 125	applications, 214	embryos, 408
materials, 122-123	data analysis, 213	germ cell apoptosis, 408-409, 411
overview, 122	digitonin extraction, 210-211	image analysis, 409
protein G Sepharose bead incubation,	flow chart, 212	materials, 407-408
123-124	materials, 209-210	overview, 407
recipes, 125	overview, 209	recipes, 412
troubleshooting, 124	plate assays, 211–213	slide preparation, 408
western blot, 124	recipes, 214–215	troubleshooting, 410–411
oligomerization assay during apoptosis	troubleshooting, 213–214	overview of techniques for study, 396–398
chemical cross-linking, 132	lysosomal membrane permeabilization assay	somatic apoptosis, 393–394, 404
disulfide linking, 132	using cytosolic activity, 206-207	Caenorhabditis elegans autophagy
materials, 131–132	Bimolecular fluorescence complementation.	apoptosis, necrosis, and cell clearance, 441
overview, 131	See Caspases	dauer development, 440
recipes, 133–134	Biological safety. See Safety	fluorescent reporters, 442
western blot, 133	Blots, cell death research publications	genes, 437–439
overview, 115–116	interpretation of images, 19	green fluorescent protein-LGG-1 for
Bax	preparation of images, 15-16	detection
activation assay overview, 116	Bovine serum albumin–gold. See Lysosomal	alternative markers, 451
blue native PAGE and antibody gel shift	membrane permeabilization assay	applications, 451
assay of conformational change		data acquisition, 450-451
and oligomerization	С	fluorescence microscopy, 449
applications, 138	Caenorhabditis elegans apoptosis	larva preparation, 448–449
cell permeabilization, 136	core pathway, 394	materials, 447-448
gel electrophoresis, 137	genetic analysis, 396	overview, 447
materials, 135-136	germline apoptosis	recipes, 452
overview, 135	physiological apoptosis, 394–395	reporter development, 449-450
recipes, 138-139	stress-induced apoptosis, 395	troubleshooting, 449
troubleshooting, 138	induction	L1 arrest after starvation, 440
conformational change in activation	genotoxic agent induction, 401, 404	longevity pathways, 440–441
flow cytometry	irradiation induction, 402	overview
applications, 129	materials, 400–401	apoptosis, 437–438
cell harvesting, 128	microscopy, 402	assays, 441–443
conformation-specific antibodies, 128	overview, 400	P granule degradation marker detection
flow cytometry, 128	recipes, 405	of embryo autophagy
materials, 127-128	RNA interference, 401, 403	applications, 457
overview, 127	troubleshooting, 402–403	data analysis, 456–457
recipes, 129	fluorescence microscopy	embryo mounting and microscopy, 455
troubleshooting, 128-129	acridine orange staining, 414–415	markers
immunoprecipitation analysis	applications, 418–419	PGL-1, 456
cell harvesting, 123	live time-course studies using	SEPA-1, 456
cell lysis, 124	fluorescent proteins, 415	SQST-1, 456
detergents, 125	materials, 413–414	materials, 454-455
materials, 122-123	overview, 413	overview, 454
overview, 122	quantitative analysis, 419	recipes, 457-458
protein G Sepharose bead incubation,	recipes, 419–420	troubleshooting, 455
123-124	RNA interference low-throughput	paternal mitochondria degradation, 441
recipes, 125	screening, 416–417	RNA interference-mediated inactivation
troubleshooting, 124	troubleshooting, 417–418	of autophagy genes
western blot, 124	RNA interference high-throughput	applications, 473–474
oligomerization assay during apoptosis	screening	data analysis, 472–473
chemical cross-linking, 132	applications, 424–425	flow chart, 469
disulfide linking, 132	data analysis, 425–426	materials, 468–469
materials, 131–132	materials, 421–422	microscopy, 470–471
overview, 131	microscopy, 423–424	overview, 468, 471–472
recipes, 133-134	overview, 421	plate preparation and incubation, 469–470
western blot, 133	plate preparation, 422–423	recipes, 474
overview, 115–116	recipes, 426	troubleshooting, 470
subcellular localization and membrane	staining, 423	western blot of LGG-1
integration analysis	troubleshooting, 424	applications, 464–465
apoptosis induction, 118	immunostaining for markers	flow chart, 460
applications, 120	acetone blocking powder	gel electrophoresis, 461–462
carbonate extraction, 119–120	preparation, 431–432	immunoblotting, 462
materials, 117–118	applications, 432–433	marker establishment and characteristics
overview, 117	gonad dissection and staining,	463–464
permeabilization of cells, 118–119	430–431	materials, 459–460
permenomenton of cens, 110 117	100 101	11141011410, 100

Caenorhabditis elegans autophagy (Continued)	recipes, 359	recipes, 241
membrane transfer, 462	substrates, 359	cytosolic cathepsin and β-N-acetyl
overview, 459	caspase-3 immunostaining	glucosaminidase activity assays
recipes, 465–466	fixation and staining, 349	applications, 214
sample preparation, 461	fluorescence microscopy, 349–350	data analysis, 213
Caspases	materials, 348–349	digitonin extraction, 210-211
activating complexes. See Apoptosome;	overview, 348	flow chart, 212
Death-inducing signaling	recipes, 351	materials, 209-210
complex	specificity, 350	overview, 209
activation pathways, 142–143, 173–174 activity assays	troubleshooting, 350	plate assays, 211–213
overview, 178–179	caspase types, 337 functions outside of cell death, 3–4	recipes, 214–215
commercial kits, 179	inhibitors, 177	troubleshooting, 213–214
cytochrome c activation assay	overview of assays, 26–27	cytosolic enzyme activity assay overview,
apoptosome activation, 183	riptosome analysis by caspase-8	206–207 CD95. See Death-inducing signaling complex
applications, 184	immunoprecipitation	Colony-forming assay, cell survival
cytosolic extract preparation, 182–183	applications, 278–279	quantification
materials, 181–182	immunoprecipitation, 275–276	adherent cells
recipes, 184–185	materials, 272-273	applications, 50
troubleshooting, 183	overview, 272	cell plating, 48–49
fluorescence well plate assay	protein sample preparation, 273-274	fixation and staining, 49
applications, 188–189	recipes, 279	materials, 47–48
incubation conditions, 187 materials, 186–187	troubleshooting, 278	overview, 47
overview, 186	western blot, 276	recipes, 50
recipes, 189	work flow, 274	hematopoietic cells on soft agar
troubleshooting, 188	single-cell imaging	applications, 53
antibody-based techniques	advantages, 142, 144 bimolecular fluorescence	cell plating, 52–53
applications, 193	complementation	colony counting, 53
cell lysis, 192	applications, 157–158	materials, 51–52
cell treatment and harvesting,	materials, 149–151	overview, 51
191-192	overview, 144–145, 149, 156–157	recipes, 53-54
flow chart, 191	recipes, 158	overview, 23
gel electrophoresis, 192	single time point analysis of caspase	CRISPR, cell death applications, 8
materials, 190–191	activation, 152–154	Crystal violet viability assay
overview, 190	time-lapse microscopy,	cell preparation, 255
recipes, 194	154-155	materials, 254
troubleshooting, 192–193	transfection with BiFC plasmid pairs,	overview, 254
inhibitor binding to active caspases applications, 198–199	151-152	recipes, 256
cell incubation and lysis, 197	troubleshooting, 156	staining and measurement, 256
flow chart, 197	fluorescence resonance energy transfer	troubleshooting, 256
materials, 196–197	applications, 168–170	Cytochrome c
overview, 196	cell number, 168	caspase activation assay
recipes, 199	cell preparation, 161–162 controls, 162–163, 168	apoptosome activation, 183 applications, 184
troubleshooting, 198	flow chart, 161	cytosolic extract preparation,
western blot, 198	image processing and data analysis,	182–183
substrate verification	164–167	materials, 181–182
applications, 203	materials, 159-160	recipes, 184–185
flow chart, 202	overview, 145-146, 159	troubleshooting, 183
gel electrophoresis, 202	recipes, 170	release assay
materials, 201–202	routine measurements, 163	applications, 88
overview, 201	sensors, 160	flow chart, 86
recipes, 203	troubleshooting, 167–168	fluorescence microscopy, 87-88
troubleshooting, 202–203	fluorescently labeled inhibitor of	incubation conditions, 86-87
caspase-3 cleavage detection in apoptotic cells	caspases, 147	materials, 85
antibody labeling, 81	fluorogenic substrates, 146–147	overview, 27, 85
applications, 82	overview, 141	recipes, 88
cell preparation, 81	rationale, 141–142	Cytospinning with rapid staining, morphological
flow cytometry, 82 materials, 80–81	substrates and cleavage motifs, 174–177	analysis of cell death
recipes, 83	Cathepsins cell death role, 178	applications, 58 cell harvesting, 56–57
caspase-8 assay. See Death-inducing signaling	lysosomal membrane permeabilization assay	cytospinning, 56
complex	cysteine cathepsin assay with fluorogenic	hematoxylin and eosin staining, 57
caspase-Glo 3/7 assay for high-throughput	substrate	materials, 55–56
screening, 493, 495–496	advantages, 240	overview, 55
cell death pathway overlap, 7–8	cell preparation, 237	recipes, 58
Drosophila	controls, 239–240	. I
activity assays	data analysis, 238–239	
fluorimetric assays, 358	incubation conditions, 237, 239	D
lysate preparation, 358	lactate dehydrogenase assay, 239	Data normalization, guidelines for publication,
materials, 357	materials, 236-237	16-17
overview, 357	overview, 226-227, 236	Death, defining for a cell, 6, 11-12

Death-inducing signaling complex (DISC)	flow chart, 366	cell density, 483
activation, isolation, and analysis	flow cytometry, 366-367	controls, 483-484
applications, 112	fluorescence microscopy, 368	data density maximization, 482
caspase-8 assay, 111-112	materials, 364–365	drug action, 482
cell lysis and treatment, 110–111	ovaries, 367–368	dynamic range, 498
flow chart for affinity purification, 109	overview, 368	initial considerations, 478-479
immunoprecipitation, 111	recipes, 369–370	limitations of assay, 484–485
materials, 107–108	staining, 366	live versus fixed analysis, 482-483
overview, 107	troubleshooting, 368–369	model system, 481–482
recipes, 112–113	ovary, 362	reproducibility, 485
SDS-PAGE and western blot, 112	Ref(2)P assays in ovaries	timing of effect, 484
troubleshooting, 112	applications, 377–378	validation, 485
overview, 95–96 DIAP1, 336–337	flow chart, 375	work flow, 484
DISC. See Death-inducing signaling complex	immunofluorescence microscopy, 374	drug development, 8–9 modes
DNA fragmentation, overview of assays, 25–26	materials, 372–373	cell suspensions versus adherent cells,
Drosophila apoptosis	ovary preparation, 374	481
acridine orange assay of dying cells	overview, 372	high-content imaging mode, 479–480
applications, 346	recipes, 378–379	plate reader mode, 480–481
embryo staining, 345	troubleshooting, 376–377	RNA interference screening. See also
larva tissue staining, 345–346	western blot, 376	Caenorhabditis elegans apoptosis
materials, 344–345		caspase-Glo 3/7 assay, 492, 495–496
overview, 344	-	cell counting with nuclear staining,
recipes, 347	E	493-494
troubleshooting, 346	Error bars, interpretation in cell death research	CellTiter-Fluor assay, 492, 495-496
caspase activity assays	publications, 19	data analysis, 494–496
fluorimetric assays, 358		high-content microscopy, 498-499
lysate preparation, 358	F	materials, 488-490
materials, 357	Fas ligand, apoptosis induction, 21	multiplex assay validation, 499-500
overview, 357	Figures, cell death research publications	overview, 488
recipes, 359	blots and gels	reverse transfection and treatment,
substrates, 359	interpretation of images, 19	490-492
caspase-3 immunostaining	preparation of images, 15–16	troubleshooting, 496–498
fixation and staining, 349	graphs	Hoechst 33342, nuclear staining
fluorescence microscopy, 349-350	interpretation, 10–11	applications, 62
materials, 348-349	preparation, 14–15	fluorescence microscopy, 62
overview, 348	image quality, 13	materials, 60–61
recipes, 351	microscopy images, 16	overview, 60
specificity, 350	statistics, 12–13	recipes, 63 staining, 61–62
troubleshooting, 350	Flow cytometry. See Annexin V and propidium	troubleshooting, 62
caspase types, 337	iodide uptake assay; Bak; Bax;	HTS. See High-throughput screening
development and metamorphosis, 335–336	Caspases; Drosophila autophagy;	1110. occ 111gh throughput screening
machinery, 336–338	Lysosomal membrane permeabi-	
overview of assays, 338	lization; Mammalian cell	I
RNA interference studies of death stimuli cell culture, 354	autophagy assays; Mitochondrial	Images, publication guidelines, 13, 15-16
cell lines, 355–356	transmembrane potential assay;	
double-stranded RNA	Propidium iodide	
cell treatment, 354–355	Fluorescence resonance energy transfer.	L
synthesis, 353–354	See Caspases	Laboratory safety. See Safety
materials, 352–353	Fluorodeoxyglucose. See Positron emission	LGG-1. See Caenorhabditis elegans autophagy
overview, 352	tomography	Life, defining for a cell, 7, 11–12
quantitative polymerase chain reaction,		LMP. See Lysosomal membrane
355	G	permeabilization
recipes, 356	Gel filtration, apoptosome, 102	Lysosomal membrane permeabilization (LMP)
total RNA preparation, 355	Gels, cell death research publications	assays
viability assays, 354	interpretation of images, 19	bovine serum albumin-gold release assay
TUNEL assay	preparation of images, 15-16	advantages, 244
applications, 342	Granzyme B, cell death role, 3, 178	data analysis, 244
fluorescence microscopy, 341-342	Graphs, cell death research publications	fixation and microscopy, 243–244
incubation conditions, 341	interpretation, 10–11	materials, 242–243
materials, 340–341	preparation, 14–15	overview, 226–227, 242
overview, 340		recipes, 246
recipes, 343	Н	tracer loading, 243
troubleshooting, 342		cysteine cathepsin assay with fluorogenic
Drosophila autophagy	Hazardous chemicals. See Safety	substrate
functions, 361	Hematoxylin and eosin staining. See	advantages, 240
LysoTracker staining	Cytospinning with rapid staining High-throughput screening (HTS)	cell preparation, 237 controls, 239–240
applications, 369 autophagy induction by amino acid	bioinformatics, 495–496	data analysis, 238–239
starvation, 365	cell death assay applications, 477–478	incubation conditions, 237, 239
cultured cells, 365–366	design of assay	lactate dehydrogenase assay, 239
** * * * * * * * * * * * * * * * * * *		, , , , , , , , , , , , , , , , , , , ,

Lysosomal membrane permeabilization (LMP)	troubleshooting, 300-301	light microscopy for differentiation of cell
assays (Continued)	immunofluorescence microscopy	death types, 23–25
materials, 236–237	applications, 295	Mitochondrial damage. See Cytochrome c
overview, 226–227, 236	cell culture and treatment, 292–293	release; Mammalian cell
recipes, 241	fixation, 294	autophagy; Mitochondrial
cytosolic cathepsin and β-N-acetyl	materials, 291-292	transmembrane potential assay
glucosaminidase activity assays	staining, 294	Mitochondrial transmembrane potential assay
applications, 214	troubleshooting, 294-295	applications, 92–93
data analysis, 213	western blot for monitoring autophagic	flow chart, 91
digitonin extraction, 210-211	flux	flow cytometry, 92
flow chart, 212	advantages, 288–289 applications, 289–290	materials, 90-91
materials, 209-210	cell treatment with inhibitor, 287	overview, 27, 90
overview, 209	data analysis, 288	TMRE staining, 91
plate assays, 211–213	flow chart, 285	
recipes, 214–215	inhibitor saturation concentration,	N
troubleshooting, 213–214	284-287	NAG. See β-N-Acetyl glucosaminidase
fluorescent dextran release assay	limitations and caveats, 289	Necroptosis
data analysis, 218	materials, 283–284	marker development, 8
dextran loading, 218	overview, 283	overview, 4–5
fluorescence microscopy, 218 materials, 216–217	protein extraction, 287	Necrosis
overview, 215	recipes, 290	annexin V and propidium iodide uptake
real-time imaging, 218–219	troubleshooting, 288	assay. See Annexin V and
troubleshooting, 218	western blot, 287–288	propidium iodide uptake assay
immunocytochemistry	p62 immunohistochemistry on paraffin sections	distinguishing from other cell death
cell treatment, 221–222		modalities. See also Annexin V and
fluorescence microscopy, 222	antibody and peptide preparation, 304–305 antigen retrieval, 305	propidium iodide uptake assay
materials, 220-221	applications, 306–307	cell lines for study, 252
overview, 220, 223	deparaffinization and rehydration, 305	crystal violet viability assay
recipes, 223	hematoxylin counterstaining, 306	cell preparation, 255
slide preparation, 222	materials, 303–304	overview, 254 materials, 254
troubleshooting, 222-223	mounting and imaging, 306	staining and measurement, 256
lysomotropic dyes to exclude	overview, 303	troubleshooting, 256
permeabilization	staining, 305–306	recipes, 256
cell preparation, 231	troubleshooting, 306	overview, 23–27
dyes, 232, 234	MAP1LC3B	riptosome analysis by caspase-8
example, 233–234	mammalian cell autophagy assays	immunoprecipitation
flow cytometry, 232	colocalization with lysosomal markers in	applications, 278–279
materials, 230–231	primary cells	immunoprecipitation, 275–276
overview, 226, 230 recipes, 235	applications, 301–302	materials, 272-273
tips, 234	data analysis, 298, 300	overview, 272
overview, 206–207, 226–227	flow cytometry, 297–299	protein sample preparation, 273-274
inducers, 226	materials, 296–297	recipes, 279
markers, 226	recipes, 302 troubleshooting, 300–301	troubleshooting, 278
programmed cell death studies, 205-206	immunofluorescence microscopy	western blot, 276
protease translocation, 205–206	applications, 295	work flow, 274
LysoTracker. See Drosophila autophagy	cell culture and treatment, 292–293	strategies, 250–252
, , , , , , , , , , , , , , , , , , , ,	fixation, 294	overview, 4–5
M	materials, 291–292	Necrostatin-1, 250
Mammalian cell autophagy	staining, 294	NETosis, overview, 5–6 Neutrophil extracellular trap. <i>See</i> NETosis
flow cytometry of mitochondrial mass, damage,	troubleshooting, 294–295	Nuclear condensation
and reactive oxygen species	western blot for monitoring autophagic	overview of assays, 23, 25, 60
cell preparation, 309–310	flux	staining. See Hoechst 33342
fixation, 310–311	advantages, 288-289	5411111g. 566 115661151 555 12
flow cytometry, 311	applications, 289-290	
materials, 308-309	cell treatment with inhibitor, 287	Р
mitochondria staining, 310	data analysis, 288	p, statistical analysis in cell death research,
overview, 308	flow chart, 285	12-13
recipes, 312	inhibitor saturation concentration,	p62
troubleshooting, 311	284–287	autophagy function, 281
inhibitors and inducers, 281	limitations and caveats, 289	Caenorhabditis elegans autophagy, 456
overview, 280–281	materials, 283–284	fluorescent reporters in UAS-GAL4 system
primary versus transformed cells, 281–282	overview, 283 protein extraction, 287	applications, 383–384
MAP1LC3B assays	recipes, 290	autophagy induction by starvation, 381
colocalization with lysosomal markers in	troubleshooting, 288	fixation and staining, 382
primary cells applications, 301–302	western blot, 287–288	flow chart, 382 materials, 380–381
data analysis, 298, 300	overview of autophagy assays, 280–281	ovary dissection, 382
flow cytometry, 297–299	Microscopy. See also specific assays	overview, 380
materials, 296–297	image preparation for cell death research	recipes, 384–385
recipes, 302	publications, 16	troubleshooting, 383

o62 (Continued)	apoptotic cell DNA content assay by cell	radiation safety, 506
genetic manipulation in ovary	cycle stage	resources, 503
applications, 390-391	applications, 73	waste disposal, 505
autophagy induction and analysis,	cell preparation, 70	Sequestosome-1. See p62
389-390	flow chart, 71	Single-cell imaging. See Caspases
germline clone generation, 387-388	flow cytometry, 71–73	Statistical analysis, cell death research, 12-13
germline expression of interfering RNA,	materials, 69–70	•
388-389	overview, 69	
materials, 386–387	recipes, 73	T
overview, 386	staining, 70–71	
		Tetramethylrhodamine ethyl ester. See
recipes, 391–392	cell death quantification	Mitochondrial transmembrane
somatic clone generation, 388	advantages and limitations, 45	potential assay
troubleshooting, 390	cell harvesting, 43	TRAIL, apoptosis induction, 21-22, 95-96
immunohistochemistry on paraffin sections	data analysis, 45	Trypan blue
for mammalian cell autophagy	flow cytometry, 44-45	assay for cell death quantification
detection	materials, 42-43	calculations, 40
antibody and peptide preparation,	propidium iodide treatment, 43	cell harvesting, 39
304-305	recipes, 46	flow chart, 39
antigen retrieval, 305	marker use for living and dead cells, 22, 42	hemocytometer counting, 39-40
applications, 306–307	tumor apoptosis assay in mice	materials, 38
deparaffinization and rehydration, 305	B-cell lymphoma transplantation in mice	recipes, 41
hematoxylin counterstaining, 306	and apoptosis induction,	trypan blue treatment, 39
materials, 303–304	331–332	
	cell lysis and staining, 332	Drosophila cell viability assay, 354
mounting and imaging, 306		uptake in dead cells, 22, 38
overview, 303	flow cytometry, 333	TUNEL assay
staining, 305–306	materials, 330–331	acridine orange assay of dying cells
troubleshooting, 306	overview, 330	applications, 346
Ref(2)P assays in <i>Drosophila</i> ovaries	recipes, 333-334	embryo staining, 345
applications, 377–378	tumor tissue collection and cell	larva tissue staining, 345-346
flow chart, 375	suspension preparation, 332	materials, 344–345
immunofluorescence microscopy, 374	PS. See Phosphatidylserine	overview, 344
materials, 372–373	Pyroptosis	recipes, 347
ovary preparation, 374	marker development, 8	troubleshooting, 346
,	overview, 4	applications, 66
overview, 372	overview, 1	B-cell lymphoma apoptosis detection
recipes, 378–379		, 1 , 1 1
troubleshooting, 376–377	R	after transplantation in mice,
western blot, 376	Radiation safety. See Safety	327–328
Perforin, cell death induction, 3	RAIDD, 142, 145, 157, 174	Drosophila apoptosis
PET. See Positron emission tomography	Rapi-Diff staining. See Cytospinning with rapid	applications, 342
Phosphatidylserine (PS)	staining	fluorescence microscopy, 341-342
annexin V binding. See Annexin V and	Reactive oxygen species. See Mammalian cell	incubation conditions, 341
propidium iodide uptake assay	, 0	materials, 340-341
overview of assays, 26	autophagy	overview, 340
Phospho-MLKL antibody, necroptosis marker, 8	Receptor-interacting protein kinase (RIPK), 177,	recipes, 343
PI. See Propidium iodide	250–252, 272, 278–279	troubleshooting, 342
PIDDosome, 142, 174	Ref(2)P. See p62	flow chart, 65
	Replicates, guidelines for publication, 17	fluorescence microscopy, 66
Positron emission tomography (PET)	RIPK. See Receptor-interacting protein kinase	± ,
hematological tumor apoptosis response	Riptosome	incubation conditions, 65–66
to drugs	analysis by caspase-8 immunoprecipitation	materials, 64–65
applications, 318	applications, 278–279	overview, 64
B-cell lymphoma transplantation in	immunoprecipitation, 275–276	recipes, 67
mice, 317	materials, 272–273	
data analysis, 318	overview, 272	
fluorodeoxyglucose administration and	protein sample preparation, 273–274	U
uptake measurement, 317–318		UAS-GAL4. See Drosophila autophagy
materials, 316–317	recipes, 279	Ultraviolet radiation-induced cell death
overview, 316	troubleshooting, 278	adherent cell culture, 35
recipes, 319	western blot, 276	Caenorhabditis elegans apoptosis, 402
solid tumor apoptosis response to drugs	work flow, 274	materials, 34–35
	necrosis role, 250-252	overview, 34, 36
applications, 323	RNA interference. See Caenorhabditis elegans	
data analysis, 323	apoptosis; Drosophila apoptosis;	radiation exposure, 35–36
fluorodeoxyglucose administration and	Drosophila autophagy; High-	recipes, 37
uptake measurement, 322	throughput screening	
materials, 320-321	0 1	***
overview, 320		W
tumor cell transplantation in mice,	S	Western blot. See Apoptosome; Bak; Bax; Blots
321-322	Safety	Caenorhabditis elegans autophag
Propidium iodide (PI)	biological safety, 506-507	Caspases; Death-inducing signal
annexin V and propidium iodide uptake	general cautions, 503–505	ing complex; Drosophila autoph
assay. See Annexin V and	hazardous chemical general properties,	agy; Mammalian cell autophagy
propidium iodide uptake assay	507–508	Riptosome
propiniani iodide uptake assay	507 500	raptosome