IMAP Assay Substrates

To facilitate the customization of the IMAP® assay to fit your needs, Molecular Devices offers a range of validated substrates and calibrators. The IMAP substrates, which have been optimized for maximum performance in FP and TR-FRET detection, are offered individually as a complement to the IMAP Screening platform. The IMAP Screening platform allows greater flexibility by providing the detection portion of the IMAP assay customized for each substrate sequence, leaving the enzymatic reaction to be determined by the researcher. You can use these substrates for the enzymes for which they were tested or as potential substrates for alternate enzymes. The following lists the peptide substrates, their amino acid sequences, part numbers, recommended Binding Solution formulation and binding incubation times[1] for FP and TR-FRET detection. Due to the greater flexibility in substrate concentration using TR-FRET detection, the substrate amounts are given now as nmoles/vial rather than dp/vial.

IMAP Peptide Substrates and Calibrators

Quantity: 8,000 (50 nmoles) or 50,000 (312.5 nmoles) data points based on 100 nM end concentration in kinase reaction[2]

IMAP Peptide Substrates and Calibrators

Product Name	Peptide Sequence[3]	Enzymes Assayed With the Substrate[4]	50 nmoles Substrate	312.5 nmoles Substrate	IMAP Progressive Binding System[1,5]				
			8,000 Data Points based on 100 nM in reaction	50,000 Data Points based on 100 nM in reaction	Detection System (TR-FRET Tb donor: 1/400 for all)	1x Buffer A (%)	1x Buffer B (%)	Binding Reagent Dilution	Minimum Binding Incubation Time
FAM-Crosstide	5FAM-GRPRTSSFAEG-COOH	Akt1, Akt2, Akt3, MSK1, MSK2, SGK1, Plk3	R7110	Inquire	FP	75	25	1/600	1 hour
					TR-FRET	40	60	1/800	overnight
FAM-EGFR-derived peptide	LVEPLTPSGEAPNQK-5FAM-COOH	JNK1, JNK2, JNK3, p38 (α,β,γ,δ isoforms)	R7129	Inquire	FP	60	40	1/1200	2 hour
					TR-FRET	30	70	1/800	overnight
FAM-p34cdc2-derived peptide	5FAM-KVEKIGEGTYGVV-NH_2	Src, Fyn, Lck, Yes, Hck, Rse	R7157	R7172	FP	75	25	1/600	1 hour
					TR-FRET	30	70	1/800	overnight
FAM-S6 Ribosomal Protein-derived peptide	5FAM-AKRRRLSSLRA-COOH	ROCK-II, Rsk1,Rsk2, Rsk3, Tab1	R7184	R7229	FP	100	0	1/400	30 minutes
					TR-FRET	70	30	1/600	3 hours
FAM-PKAtide	5FAM-GRTGRRNSI-NH_2	Aurora A‡, PKA*, PKG	R7250	R7255	FP	100	0	1/400	30 minutes
					TR-FRET	80	20	1/800	1 hour

IMAP Peptide Substrates and Calibrators (continued)

Product Name	Peptide Sequence[3]	Enzymes Assayed With the Substrate[4]	50 nmoles Substrate	312.5 nmoles Substrate	IMAP Progressive Binding System[1,5]				
			8,000 Data Points based on 100 nM in reaction	50,000 Data Points based on 100 nM in reaction	Detection System (TR-FRET Tb donor: 1/400 for all)	1x Buffer A (%)	1x Buffer B (%)	Binding Reagent Dilution	Minimum Binding Incubation Time
FAM-Histone H1-derived peptide	5FAM-GGGPATPKKAKKL-COOH	CDK1/Cyclin B, CDK2/Cyclin A, CDK2/CyclinE, CDK3/CyclinE, CDK5/p25, CDK5/p35, CDK6/Cyclin D3	R7252	R7257	FP	100	0	1/400	30 minutes
					TR-FRET	80	20	1/600	1 hour
FAM-IκBα-derived peptide	5FAM-GRHDSGLDSMK-NH_2	IKKβ*, IKKα	R7254	Inquire	FP	75	25	1/600	1 hour
					TR-FRET	30	70	1/800	overnight
FAM-Erktide 0.1% BSA rxn buffer[5]	IPTTPITTTYFFFK-5FAM-COOH	Erk1, Erk2, p38 (α,β,γ,δ isoforms)	R7292	Inquire	FP	60	40	1/1200	4 hours
					TR-FRET	Not tested			
FAM-Erktide 0.01% Tween-20 rxn buffer[5]	IPTTPITTTYFFFK-5FAM-COOH	Erk1‡, Erk2‡, p38 (α,β,γ,δ isoforms)‡	R7292	Inquire	FP	75	25	1/600	1 hour
					TR-FRET	70	30	1/600	3 hours
TAMRA-EGFR-derived peptide	LVEPLTPSGEAPNQK-5TAMRA-NH_2	JNK1, JNK2, JNK3, p38 (α,β,γ,δ isoforms)	R7307	Inquire	FP	75	25	1/600	1 hour
					TR-FRET	60	40	1/800	4 hours
FAM-CK1tide 0.1% BSA rxn buffer[5]	5FAM-HAAIGDDDDAYSITA-NH_2	CK1, CK1δ	R7311	R7312	FP	30	70	1/1500	overnight
					TR-FRET	Not tested			

IMAP Peptide Substrates and Calibrators (continued)

Product Name	Peptide Sequence[3]	Enzymes Assayed With the Substrate[4]	50 nmoles Substrate	312.5 nmoles Substrate	IMAP Progressive Binding System[1,5]				
			8,000 Data Points based on 100 nM in reaction	50,000 Data Points based on 100 nM in reaction	Detection System (TR-FRET Tb donor: 1/400 for all)	1x Buffer A (%)	1x Buffer B (%)	Binding Reagent Dilution	Minimum Binding Incubation Time
FAM-CK1tide 0.01% Tween-20 rxn buffer[5]	5FAM-HAAIGDDDDAYSITA-NH_2	CK1, CK1δ	R7311	R7312	FP	25	75 and 0.03% BSA[6]	1/1500	4 hours
					TR-FRET	0	100	1/1000	overnight
TAMRA-PKAtide	5TAMRA-GRTGRRNSI-COOH	PKA, PKG	R7313	Inquire	FP	100	0	1/400	30 minutes
					TR-FRET	70	30	1/600	3 hours
FAM-EGFR-derived phosphopeptide	LVEPL-pT-PSGEAPNQK-5FAM-COOH	Calibrator	R7319	Inquire	FP	75	25	1/600	1 hour
					TR-FRET	30	70	1/800	overnight
TAMRA-CDK7tide	5TAMRA-YSPTSPSYSPTSPSYSPTSPS-COOH	CDK7	R7352	Inquire	FP	100	0	1/400	30 minutes
					TR-FRET	70	30	1/600	3 hours
TAMRA-Rostide	5TAMRA-KKKSPGEYVNIEFG-NH_2	Ros, Met	Inquire	R7383	FP	75	25	1/600	1 hour
					TR-FRET	30	70	1/600	overnight
FAM-p38tide 0.01% Tween-20 rxn buffer[5]	5FAM-IPTTPITTTYFFFK-NH_2	Erk1, Erk2, p38 (α,β,γ,δ isoforms)	R7434	Inquire	FP	75	25	1/600	1 hour
					TR-FRET	70	30	1/600	3 hours
FAM- CaM KI-ADR/Synapsin Chimera peptide	5FAM-LKKLRRRSDANF-NH_2	CaMKI	RP7005	Inquire	FP				
					TR-FRET				

IMAP Peptide Substrates and Calibrators (continued)

Product Name	Peptide Sequence[3]	Enzymes Assayed With the Substrate[4]	50 nmoles Substrate	312.5 nmoles Substrate	IMAP Progressive Binding System[1,5]				
			8,000 Data Points based on 100 nM in reaction	50,000 Data Points based on 100 nM in reaction	Detection System (TR-FRET Tb donor: 1/400 for all)	1x Buffer A (%)	1x Buffer B (%)	Binding Reagent Dilution	Minimum Binding Incubation Time
FAM-PKCε pseudosubstrate (A to S mutated) derived peptide	5FAM-RKRQGSVRRRVH-OH	PKC	RP7030	Inquire	FP				
					TR-FRET				
FAM-PKCα pseudosubstrate derived peptide	5FAM-RFARKGSLRQKNV-COOH	PKCζ	RP7032	Inquire	FP	100	0	1/400	30 minutes
					TR-FRET	70	30	1/400	3 hours
FAM-IP3R-derived peptide	5FAM-GRRESLTSFG-NH_2	PKA, PKG	RP7035	Inquire	FP	75	25	1/600	1 hour
					TR-FRET	45	55	1/600	overnight
FAM-Alternate Syntide2	5FAM-PLSRTLSVSSLPGL-NH_2	c-TAK	RP7045	Inquire	FP	100	0	1/400	30 minutes
					TR-FRET	80	20	1/400	1 hour
FAM-CREBtide	5FAM-GEILSRRPSYRK-NH_2	MSK1‡	RP7046	Inquire	FP	100	0	1/400	30 minutes
					TR-FRET	80	20	1/400	1 hour
FAM-CDK7tide	5-FAM-YSPTSPSYSPTSPSYSPTSPS-OH	TKL, STE, CK1, CMGC	RP7116	RP7616	FP				
					TR-FRET				
FAM-p53 derived peptide	5-FAM-EPPQSQEAFADLWK-NH_2	TKL, STE, CK1, CMGC	RP7119	Inquire	FP				
					TR-FRET				
FAM-DYRKtide	5-FAM-RRRFRPASPLRGPPK-OH	Dyrk1A, TKL, STE, CK1, CMGC	RP7120	Inquire	FP				
					TR-FRET				

IMAP Peptide Substrates and Calibrators (continued)

Product Name	Peptide Sequence[3]	Enzymes Assayed With the Substrate[4]	50 nmoles Substrate	312.5 nmoles Substrate	IMAP Progressive Binding System[1,5]				
			8,000 Data Points based on 100 nM in reaction	50,000 Data Points based on 100 nM in reaction	Detection System (TR-FRET Tb donor: 1/400 for all)	1x Buffer A (%)	1x Buffer B (%)	Binding Reagent Dilution	Minimum Binding Incubation Time
FAM-MBP derived peptide	5-FAM-ATGPLSPGPFGRR-OH	Erk2, TKL, STE, CK1, CMGC	RP7123	Inquire	FP				
					TR-FRET				
FAM-p44 derived peptide(aa200-218)	5-FAM-FLTEYVATRWYRAPEIMLN-NH_2	MAPK, TKL, STE, CK1, CMGC	RP7131	Inquire	FP				
					TR-FRET				
FAM-extended PLMderived peptide 0.01% Tween-20 rxn buffer[5]	5FAM-GTFRSSIRRLSTRRR-OH	Nek2, IRAK4‡	RP7140	RP7640	FP	100	0	1/400	30 minutes
					TR-FRET	70	30	1/400	3 hours
FAM-RS domain derived peptide 0.01% Tween-20 rxn buffer[5]	5FAM-GRSRSRSRSR-OH	SPRK1, IRAK4	RP7153	Inquire	FP	100	0	1/400	30 minutes
					TR-FRET	40	60	1/600	overnight
FAM-PKCε pseudosubstrate derived peptide	5FAM-ERMRPRKRQGSVRRRV-NH_2	PKCγ, Pim1	Inquire	RP7548	FP	85	15	1/400	30 minutes
					TR-FRET	70	30	1/400	3 hours
FAM-generic Ser/Thr protein kinases substrate peptide	5-FAM-KKRKSSLRRWSPLTPRQMSFDC-NH_2	PKA, PKC, CAMK, MAPK, TKL, STE, CK1, CMGC	Inquire	RP7609	FP				
					TR-FRET				

‡ sequence resulting in the most sensitive assay for enzymes with several possible substrates

Notes

1. The Binding Buffer conditions are a starting point for IMAP assay development. They were optimized for FP detection using 100 nM substrate in IMAP reaction buffer with BSA (containing 1 mM DTT) in the presence of 100 µM ATP unless stated otherwise. For TR-FRET detection the same DTT and ATP in 0.01% Tween containing buffer was used. If you use alternative reaction conditions, you should do a quick background check of the substrate.
2. Number of test points is based on a typical 384-well microplate assay consisting of a 20 µL kinase reaction and 60 µL of Binding Solution. Using FP detection, the most common substrate concentration is 100 nM. This parameter is far more flexible using TR-FRET detection.
3. pX = phosphorylated on X residue, 5FAM = 5-carboxyfluorescein , 5TAMRA = 5-carboxytetramethylrhodamine, -NH_2 = C-terminal amide, -COOH = C-terminal free acid.
4. An IMAP response of ΔmP >100 mP has been achieved with the substrate for any enzyme listed in this column. You can use Phosphopeptides denoted as calibrator to calibrate the IMAP response for the corresponding non-phosphorylated form.
5. 1x BSA reaction buffer : 10 mM Tris-HCl, 10 mM $MgCl_2$, 0.1% BSA, 0.05% NaN_3, pH 7.2.
   1x Tween reaction buffer: 10 mM Tris-HCl, 10 mM $MgCl_2$, 0.01% Tween-20, 0.05% NaN_3, pH 7.2.
6. You can use any commercially available BSA. Phosphate-free BSA of ^{3} 98% purity is recommended.

These tables are a reference for the name and sequence of the substrates provided with Molecular Devices Reagent Kits. You can use these substrates with the IMAP Screening Express for assay development using proprietary enzymes.

All substrates are guaranteed to have 90% or greater purity by HPLC prior to lyophilization. All of these substrates have been verified for use with the IMAP platform.

Starting Conditions to Optimize Progressive Binding Solutions for FP Detection

Number of -COOHs in Peptide	Progressive Binding Buffer A	Progressive Binding Buffer B	Progressive Binding Dilution	Binding Incubation Time (minimum)
None or 1	100%	0%	1/400	30 minutes
2 or 3	75%	25%	1/600	1 hour
4	40%	60%	1/1200	2 hours
5 or more	25%	75%	1/2500	5 hours or more depending on the number of acidic residues in the peptide

Starting Conditions for Optimization of Progressive Binding Solutions for TR-FRET Detection

Number of -COOHs in peptide	Progressive Binding Buffer A	Progressive Binding Buffer B	Progressive Binding Reagent Dilution	Binding Incubation time (minimum)
None	80%	20%	1/600	30 minutes
1	70%	30%	1/600	2 hours
2	40%	60%	1/800	4 hours
3	30%	70%	1/800	5 hours or more*
4	0%	100%	1/1000	5 hours or more*
5 or more	0%	140%**	1/1000	5 hours or more*

* depending on number of acidic residues in peptide.

** For peptide substrates that require greater than 100% 1x Buffer B, you must use a >1x stock, for example, 160% 1x Buffer B = 1.6x Buffer B. Using the 5x stock, make a 1: 3.13 dilution.

Phosphodiesterase Substrates

Phosphodiesterase Substrates

Product Name	Peptide Sequence[2,3]	20 nmoles Substrate	120 nmoles Substrate	IMAP Progressive Binding System[1,5]				
		8,000 Data Points[1] based on 100 nM in reaction	50,000 Data Points[1] based on 100 nM in reaction	Detection System (TR-FRET Tb donor: 1/400 for all)	1x Buffer A (%)	1x Buffer B (%)	Binding Reagent Dilution	Minimum Binding Incubation Time
cAMP PDE substrate[4]	(FAM)-cyclic-(3',5')-AMP	R7505	R7506	FP	85	15	1/600	1 hour
				TR-FRET	70	30	1/800	3 hour
cGMP PDE substrate[4]	(FAM)-cyclic-(3',5')-GMP	R7507	R7508	FP	75	25	1/600	1 hour
				TR-FRET	60	40	1/800	4 hour

Notes

1. The Binding Buffer conditions are a starting point for IMAP assay development. They were optimized for FP detection using 100 nM substrate in IMAP reaction buffer with BSA (containing 1 mM DTT) in the presence of 100 µM ATP unless stated otherwise. For TR-FRET detection the same DTT and ATP in 0.01% Tween containing buffer was used. If you use alternative reaction conditions, you should do a quick background check of the substrate.
2. FAM = fluorescein; phosphodiesterase substrates are supplied as a 100 µM solution in 0.1% acetic acid.
3. pX = phosphorylated on X residue, 5FAM = 5-carboxyfluorescein , 5TAMRA = 5-carboxytetramethylrhodamine, -NH_2 = C-terminal amide, -COOH = C-terminal free acid.
4. An IMAP response of ΔmP >100 mP has been achieved with the substrate for any enzyme listed in this column. You can use Phosphopeptides denoted as calibrator to calibrate the IMAP response for the corresponding non-phosphorylated form.
5. 1x BSA reaction buffer : 10 mM Tris-HCl, 10 mM $MgCl_2$, 0.1% BSA, 0.05% NaN_3, pH 7.2.
   1x Tween reaction buffer: 10 mM Tris-HCl, 10 mM $MgCl_2$, 0.01% Tween-20, 0.05% NaN_3, pH 7.2.

Protocols

These protocols are for lyophilized peptide substrates. Use substrates according to the assay protocols supplied with the kit.

IMAP Peptide Substrate Reconstitution Protocol for 50 nmoles vial (8000 test points at 100 nM)

1. Reconstitute the 50 nmoles lyophilized substrate in 2.5 mL of 1x IMAP Reaction Buffer (see the *Screening Express and IPP* document) to make a 20 µM fluorescent substrate solution.
2. Vortex gently and invert vial to make sure that all of the lyophilized substrate goes into solution.
3. Prepare the substrate working solution for the IMAP assay. For example, if you use a final reaction concentration of 100 nM substrate and want to add 5 µL of substrate solution per 20 µL reaction, then make a 4x working stock of 400 nM. (For 8000 x 20 µL reactions, this would be 800 µL of the 20 µM substrate solution plus 39.2 mL of Complete Reaction Buffer to make 40 mL of 400 nM substrate. The complete reaction buffer may contain 1 mM DTT and other additions as directed by enzyme needs.)

IMAP Peptide Substrate Reconstitution Protocol for 312.5 nmoles (50,000 test points at 100 nM)

1. Reconstitute the 312.5 nmoles lyophilized substrate in 3.125 mL of 1x IMAP Reaction Buffer (see the *Screening Express and IPP* document) to make a 100 µM fluorescent substrate solution.
2. Vortex gently and invert vial to make sure that all of the lyophilized substrate goes into solution.
3. Make a further 1/5 dilution of the substrate solution if you prefer a 20 µM stock solution. For example, add 12.5 mL 1x IMAP Reaction Buffer to the 3.1 mL of 100 µM substrate to make 15.6 mL of the 20 µM substrate solution. Vortex gently to mix.
4. Prepare the substrate working solution for the IMAP assay. For example, if you use a final reaction concentration of 100 nM substrate and want to add 5 µL of substrate solution per 20 µL reaction, then make a 4x working stock of 400 nM. For 50,000 x 20 µL reactions, this would be 5 mL of the 20 µM substrate solution plus 245 mL of Complete Reaction Buffer to make 250 mL of 400 nM substrate. The Complete Reaction Buffer may be 1x IMAP Reaction Buffer that contains 1 mM DTT and other additions as directed by enzyme requirements .

NOTES

- Although most IMAP substrates are stable for up to two weeks at 4°C after reconstitution, you should aliquot any unused portion and store at -20°C. Avoid repeated freeze/thaw cycles
- Reconstituting the 50 nmoles substrate in 2.5 mL buffer results in 1% BSA in this stock from the lyophilization and stabilization procedure. Running the assay at 100 nM substrate results in 0.005% BSA, which is very low and has been shown not to influence the assay results. If you run the assay at higher substrate concentrations, the concentration of BSA increases proportionally. BSA can bind to hydrophobic compounds and thus reduce their active concentration in the reaction which can lead to a right shift of IC50's. Consider this when you choose the reaction buffer. Most IMAP buffer kits come with a reaction buffer that contains BSA and a reaction buffer that contains a Tween. As an alternative, you can use the 312.5 nmole/vial size, which is reconstituted to 100 µM with 1% residual BSA which results in a 5x lower residual BSA concentration as compared to the 50 nmoles size.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website—support.moleculardevices.com/—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Please have the instrument serial number (on the rear of the instrument), or reagent kit lot number, and any related sample data files available when you call.

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