QUANTITATIVE PCR

Quantitative PCR Product Listing

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SYBR® Green JumpStart Taq ReadyMix

Real time detection in a complete reagent

SYBR Green JumpStart Taq ReadyMix for Quantitative PCR combines the advantages of a hot start enzyme with a ready-to-use mix, making it the ideal choice for high throughput, quantitative PCR. The ReadyMix includes the fluorescent dye SYBR Green I, JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides and buffer in an optimized 2× concentrate.

SYBR Green JumpStart Taq ReadyMix is recommended for single product real time amplification experiments. It may also be used for evaluation of primer sequences prior to manufacture of fluorescent-labeled primers. Fluorescent-labeled primers are not recommended for use with SYBR Green I dye.

SYBR Green I, a commonly used fluorescent DNA binding dye, binds all double-stranded DNA and thus product accumulation is detected by measuring the increase in fluorescence throughout the cycle. SYBR Green I has an excitation and emission maxima of 494 nm and 521 nm, respectively.

JumpStart Taq Polymerase is an antibody inactivated hot start enzyme designed to minimize non-specific amplification while increasing target yield resulting in more accurate Ct values and improved standard curve for absolute sample quantitation.

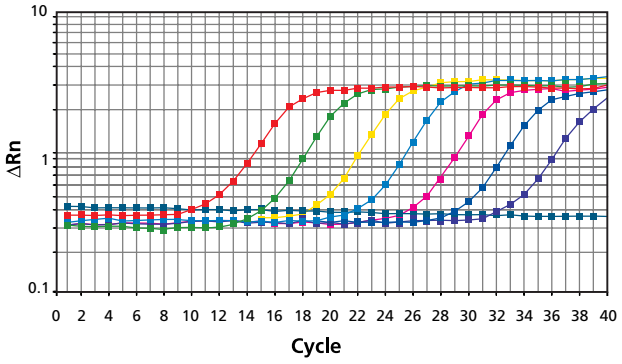
To prepare a reaction, add 25 µl of ReadyMix to primers, template and water for a final reaction volume of 50 µl.

Sigma's Reference Dye for Quantitative PCR is included with this ReadyMix for normalization of the reaction data. The dye has a maximum excitation of 586 nm, and a maximum emission of 605 nm. The instrument settings for ROX Reference Dye are suitable for the measurement of the Reference Dye for Quantitative PCR.

Features and Benefits

- Minimize non-specific amplification while increasing target yield and specificity
- Delivers the benefits of antibody inactivated hot start PCR with SYBR Green detection
- Ideal for high throughput applications; only primers and template are required
- SYBR Green JumpStart Taq ReadyMixes for SYBR based QPCR are compatible with tube- and plate-based instruments

Ct values for the Lambda Amplicon Using SYBR Green JumpStart Taq ReadyMix



Quantitative PCR (QPCR) was performed on pBac-2cp. Initial template copy number was 10^6 and was diluted 10-fold in subsequent wells. Threshold cycles (Ct) were determined using the ABI PRISM 7700 Sequence Detection software, and were found to be 15.304 (10^6), 18.848 (10^5), 22.883 (10^4), 26.208 (10^3), 29.821 (10^2), 33.398 (10^1), 37.038 (10^0), and 40 (0).

Components: SYBR Green JumpStart Taq ReadyMix
Reference Dye for Quantitative PCR
Separate Vial of 25 mM $MgCl_2$ Included in S5193

Unit definition: One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

Concentration: 1.5 units per reaction (50 µl reaction volume)

Storage: –20 °C
Shipped in wet ice

Cat. No.	Product Description	Quantity
S4438	SYBR Green JumpStart Taq ReadyMix	20 reactions 100 reactions 500 reactions
S5193	SYBR Green JumpStart Taq ReadyMix without $MgCl_2$	100 reactions 400 reactions

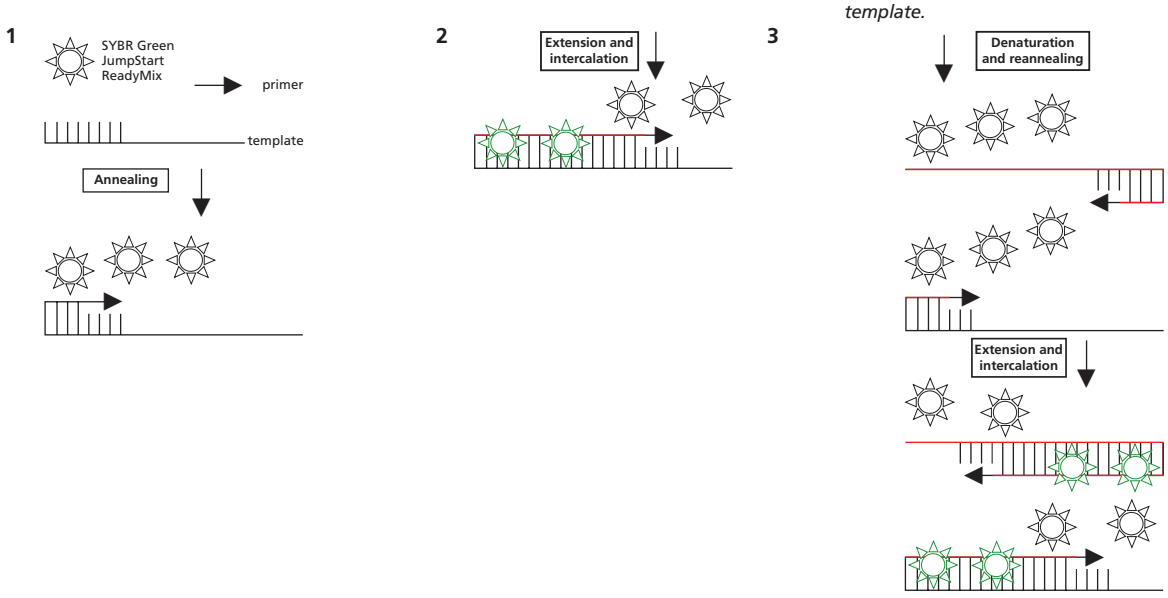
QUANTITATIVE PCR

Mechanism of SYBR Green I in QPCR

1) Set up your PCR reaction with the SYBR Green JumpStart Taq ReadyMix. The mix contains enzyme, dNTPs, buffer and SYBR Green I dye. Just add your primers and template.

2) As the reaction progresses, double-stranded products are generated. The SYBR Green I dye intercalates into these products and begins to fluoresce.

3) When enough products have accumulated the fluorescence rises above background. This is called the threshold cycle or Ct. The Ct value is used to quantify the starting amount of template.



SYBR® Green JumpStart™ Taq ReadyMix™

Capillary Formulation

SYBR Green JumpStart Taq ReadyMix, Capillary formulation combines the advantages of a hot start enzyme, JumpStart Taq, in a 2× concentrate ReadyMix specifically designed for use with capillary instruments, such as the Roche LightCycler® real-time thermal cycler. SYBR Green JumpStart Taq ReadyMix is an optimized formulation containing SYBR Green I dye, JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides, buffer and stabilizers.

SYBR Green JumpStart Taq ReadyMix is recommended for single product real time amplification experiments. Fluorescent-labeled probes are not recommended for use with SYBR Green I dye.

SYBR Green I binds all double-stranded DNA and detection is monitored by measuring the increase in fluorescence throughout the cycle.

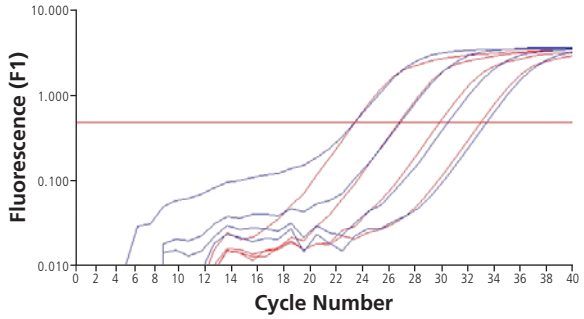
JumpStart Taq Polymerase, an antibody inactivated hot start enzyme, is designed to minimize non-specific amplification while increasing target yield resulting in more accurate Ct values and an improved standard curve for absolute sample quantitation.

To prepare a reaction, 10 µl of ReadyMix is added to primers, template and water for a final reaction volume of 20 µl. Once the reaction has reached 70 °C, the complex dissociates and the polymerase becomes fully active. No special preparations or protocol changes are required.

Features and Benefits

- Minimizes non-specific amplification while increasing target yield and specificity
- Delivers the benefits of antibody inactivated hot start PCR with SYBR Green detection in a ReadyMix specifically designed for capillary-based instruments; only primers and template are required
- SYBR Green JumpStart Taq ReadyMixes for SYBR based QPCR are formulated with $MgCl_2$ or packaged with a separate vial for ease of optimization

Efficient and Sensitive Amplification with Human Genomic DNA



Quantitative PCR was performed on human genomic DNA using the Roche LightCycler. The template was diluted 10-fold in subsequent wells with concentrations of 30 ng to 30 pg. Sigma's SYBR Green Taq ReadyMix, capillary formulation (in red) has comparable sensitivity and efficiency to supplier R's SYBR Green master mix (in blue).

Components: SYBR Green JumpStart Taq ReadyMix
Reference Dye for Quantitative PCR
Separate Vial of 25 mM $MgCl_2$ Included in S5193

Unit definition: One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

Storage: –20 °C
Shipped in wet ice

Cat. No.	Product Description	Quantity
S1816	SYBR Green Taq ReadyMix, Capillary Formulation	200 reactions 100 reactions 400 reactions
S5193	SYBR Green JumpStart Taq ReadyMix without $MgCl_2$	100 reactions 400 reactions

QUANTITATIVE PCR

JumpStart™ Taq ReadyMix™ for Quantitative PCR

For probe-specific real time PCR applications

JumpStart Taq ReadyMix for Quantitative PCR combines the advantages of a hot start enzyme with a ready-to-use mix for high throughput, quantitative PCR (QPCR). It does not contain a fluorescent detection method, which makes it compatible for use with many formats. Dual-labeled probes, Molecular Beacons, or double-stranded binding dyes such as SYBR Green I dye can all be individually optimized for use with this ReadyMix.

The ReadyMix contains JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides and buffer in an optimized 2× concentrate. JumpStart Taq Polymerase, an antibody inactivated hot start enzyme, is designed to minimize non-specific amplification while increasing target yield. Unlike other hot start methods (i.e. chemical inactivation), JumpStart Taq Polymerase does not require a pre-incubation step prior to cycling because polymerase activity is fully restored during the first denaturation cycle of the PCR reaction. To prepare a reaction, add 25 µl of the ReadyMix to primers, template, detection component and water for a total reaction volume of 50 µl.

Sigma's Reference Dye for Quantitative PCR is included with this ReadyMix for normalization of the reaction data. The dye has a maximum excitation of 586 nm, and a maximum emission of 605 nm. The instrument settings for ROX reference dye are suitable for the measurement of the Reference Dye for Quantitative PCR. A tube of 25 mM $MgCl_2$ is provided for easy optimization of the QPCR reaction.

Features and Benefits

- Minimize non-specific amplification while increasing target yield and specificity, both of which result in lower, more accurate Ct values. Also available with dUTP (Catalog Number D9191) to prevent carry-over contamination
- Compatible with a variety of fluorescent detection methods including dual-labeled probes and Molecular Beacons, recommended for multiplex comparative studies
- Designed for use with either plate/tube real time thermal cyclers or capillary instruments

Components: JumpStart Taq ReadyMix
25 mM $MgCl_2$
Reference Dye for Quantitative PCR

Unit definition: One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

Concentration: 1.5 units per reaction (50 µl reaction volume)

Storage: –20 °C
Shipped in wet ice

Cat. No.	Product Description	Quantity
D7440	JumpStart Taq ReadyMix for Quantitative PCR	100 reactions 400 reactions

Superior Amplification Efficiency with Lower Ct

Figure 1

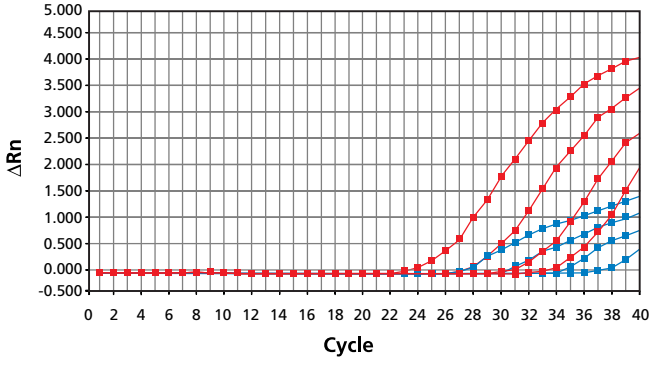
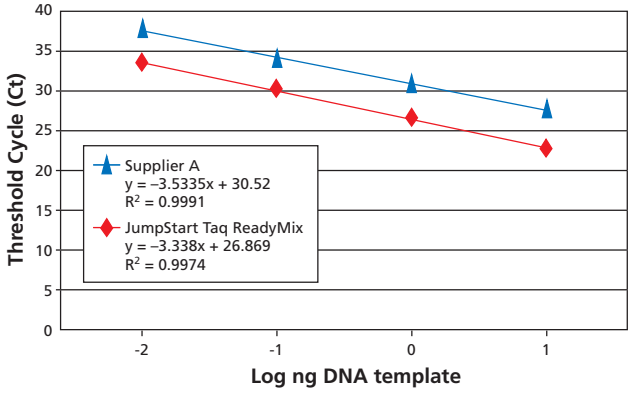


Figure 2



Quantitative PCR was performed on human genomic DNA. The template was diluted 10-fold in subsequent wells; concentrations were 10 ng, 1 ng, 0.1 ng and 0.01 ng. A TaqMan probe and primers specific for a 250 bp PCR product of the β-actin gene were used with Sigma's JumpStart Taq ReadyMix for Quantitative PCR or a master mix from Supplier A. Final magnesium concentration was adjusted to 3.5 mM. Thermal cycling conditions were those recommended by Supplier A. The JumpStart Taq ReadyMix (in red) has better amplification efficiency (Figure 1), resulting in lower Ct values than Supplier A (in blue) (Figure 2).

QUANTITATIVE PCR

JumpStart™ Taq ReadyMix™ with dUTP

JumpStart Taq ReadyMix with dUTP for Quantitative PCR Kit provides the performance enhancements of our JumpStart Taq Antibody for hot start in a convenient, easy-to-use reaction mixture. The mix has no added dyes, making it ideal for performing high throughput quantitative PCR (QPCR) methods that rely on a fluorescent probe.

The ReadyMix has incorporated dUTP in place of TTP to facilitate carryover prevention. This nucleotide mixture provides the ability to eliminate contaminating PCR products by use of a Uracil-DNA glycosylase (UNG). The ready-to-use mixture of JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides and reaction buffer is provided in a 2× concentrate. Simply add 25 µL of the 2× mix DNA template, primers, fluorescent probe, UNG, and water. At room temperature, the JumpStart Taq antibody inactivates the DNA polymerase. However, during the first denaturation step of the cycling process, the complex dissociates and the polymerase becomes fully active.

Features and Benefits

- The ideal ReadyMix for high throughput, quantitative PCR applications where carryover contamination is a concern
- The hot start mechanism using JumpStart Taq antibody prevents non-specific product formation. Assembled PCR reactions can remain at room temperature for up to 2 hours without compromising performance
- The incorporation of dUTP provides the ability to eliminate contaminating PCR products from earlier reactions with uracil-DNA. Use of uracil-DNA glycosylase (UNG) prevents carryover contamination

Components: JumpStart Taq ReadyMix with dUTP
25 mM $MgCl_2$
Reference Dye for Quantitative PCR

Unit definition: One unit incorporates 10 nmol of total dNTPs into acid-precipitable DNA in 30 min at 74 °C

Concentration: 2.5 units per reaction (50 µl reaction volume)

Storage: –20 °C
Shipped in wet ice

Cat. No.	Product Description	Quantity
D9191	JumpStart Taq ReadyMix with dUTP	100 reactions 400 reactions

QUANTITATIVE PCR

SYBR® Green Quantitative RT-PCR Kit

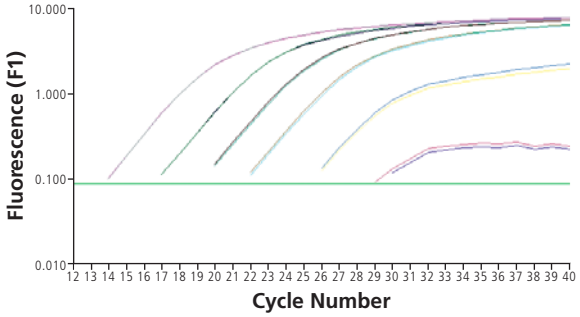
The SYBR Green Quantitative RT-PCR Kit combines eAMV Reverse Transcriptase, JumpStart Taq DNA Polymerase and SYBR Green I fluorescent dye in a one-step RT-PCR kit designed for the quantitative analysis of gene expression. eAMV has the ability to transcribe through difficult secondary structure at elevated temperatures (up to 65 °C). The ReadyMix includes SYBR Green I dye, JumpStart Taq DNA Polymerase, 99% pure deoxynucleotides, buffer and stabilizers, and is provided as a 2× concentrate for convenience. JumpStart Taq DNA Polymerase uses JumpStart Taq antibody to inactivate the enzyme below 70 °C, preventing primer dimers and non-specific product formation. The double-stranded DNA-specific fluorescent SYBR Green I reporter has high sensitivity, is easy to use and is less expensive than sequence-specific fluorescent probes.

The SYBR Green Quantitative RT-PCR Kit delivers high specificity and reproducibility. Since SYBR Green I dye will detect all non-specific quantitative RT-PCR product formation, well-designed primers are recommended for this system to ensure the highest possible specificity. The kit has been optimized for use with both plate/tube real time instruments and with the Roche LightCycler capillary instrument. A reference dye is provided in a separate vial to be used in ABI Detection Systems.

Features and Benefits

- eAMV Reverse Transcriptase has the ability to transcribe through difficult secondary structures at elevated temperature (up to 65 °C)
- Minimize non-specific amplification while increasing target yield and specificity, both of which result in lower, more accurate Ct values
- SYBR based detection allows quantitation of all double-stranded DNA. Specificity is greatly enhanced by the incorporation of JumpStart Taq. Optimized to help you achieve superior results, our eAMV RT-PCR Kit is compatible with tube, plate, and capillary-based instruments

Sensitive Quantitative RT-PCR Using Sigma's SYBR Green Quantitative RT-PCR Kit



Quantitative SYBR Green RT-PCR was performed in duplicate on human total RNA from cell line HeLa-S3. The total RNA was diluted 10-fold in subsequent capillaries with concentrations of 500 ng to 5 pg. Primers specific for a β-actin 187 bp RT-PCR product were used for amplification.

Components: SYBR Green Taq ReadyMix for Quantitative RT-PCR
eAMV Reverse Transcriptase
10× PCR Buffer
25 mM $MgCl_2$
Reference Dye for Quantitative PCR

Storage: –20 °C
Shipped in wet ice

Cat. No.	Product Description	Quantity
QR0100	SYBR Green Quantitative RT-PCR Kit	1 kit

Quantitative Reverse Transcriptase PCR ReadyMix for Probe-Based Applications

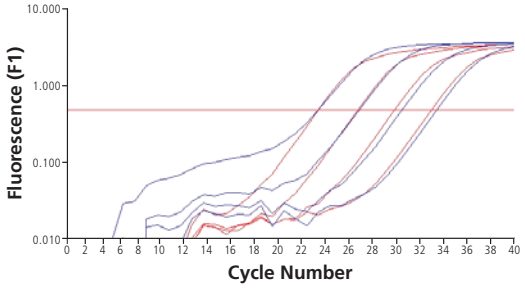
Quantitative Reverse Transcriptase PCR (QRT-PCR) provides a highly sensitive method for the quantitative analysis of gene expression. Sigma's QRT-PCR ReadyMix combines the advantages of Enhanced Avian Reverse Transcriptase (eAMV RT) and JumpStart Taq with a ready-to-use mix specifically designed for probe-based QRT-PCR. Sigma's QRT-PCR ReadyMix is specially formulated to help you achieve superior results regardless of difficult secondary structure or chosen fluorescent detection chemistry.

QRT-PCR ReadyMix for probe-based applications is a 2× concentrate blend of JumpStart Taq, 99% pure dNTPs, buffer, glass passivator, and stabilizers. The ReadyMix is also packaged with a separate vial of eAMV RT, 25 mM $MgCl_2$, 10× PCR Buffer, and Reference Dye for normalization of the reaction data.

Features and Benefits

- eAMV Reverse Transcriptase has the ability to transcribe through difficult secondary structures at elevated temperature (up to 65 °C)
- Minimize non-specific amplification while increasing target yield and specificity, both of which result in lower, more accurate Ct values
- Compatible with a variety of fluorescent detection methods including dual-labeled probes and Molecular Beacons, Sigma's ReadyMix is also formulated for use on tube, plate, and capillary-based instruments

Superior Sensitivity and Specificity with Sigma's qRT-PCR ReadyMix



Quantitative RT-PCR ReadyMix was performed in duplicate on total RNA from the HeLa S3 cell line. Total RNA was DNase treated and diluted 10-fold in subsequent capillaries. Forward and reverse primers specific for c-Myc were used for amplification. Enhanced Avian Reverse Transcriptase was diluted 1:20. Measurements were made using the Roche LightCycler®.

Components: Probe-based QRT-PCR ReadyMix
eAMV Reverse Transcriptase
10× PCR Buffer
25 mM $MgCl_2$
Reference Dye for Quantitative PCR

Storage: –20 °C

Cat. No.	Product Description	Quantity
QR0200	QRT-PCR ReadyMix for probe-based applications Sufficient for 100-50 µl reactions	1 kit

QUANTITATIVE PCR

Reference Dye for Quantitative PCR

Reference Dye is used for normalization of reaction data when using SYBR Green I dye, Molecular Beacons, or dual-labeled probe chemistries for real time detection. Instrument settings for ROX reference dye are suitable for the measurement of Reference Dye for Quantitative PCR.

0.3 ml solution is sufficient for a minimum of 600 reactions.

Storage: 2-8 °C

Cat. No.	Product Description	Quantity
R4526	Reference Dye for Quantitative PCR	0.3 ml 5.0 ml

PCR Plate Detection Kit

Perform solid-phase capture and sequence-specific detection of PCR products in an easy-to-use, automatable format, and allele-specific hybridization for the detection and genotyping of point mutations. PCR amplification is carried out with one 5′-biotinylated primer and one unlabeled primer. The amplified products are immobilized in streptavidin-coated strip-well plates. The non-biotinylated strand is removed by sodium hydroxide denaturation, and the biotinylated strand is hybridized to a sequence-specific fluorescein-labeled probe. After a wash step, the probe is detected with a peroxidase-conjugated anti-fluorescein antibody and the chromogenic peroxidase substrate TMB. The assay can be completed in 2.5 hours.

Features and Benefits

- 10-100× more sensitive than standard gel electrophoresis and allows discrimination between PCR product levels over a 2-2.5 log range of concentration. Unique PlateHyb hybridization buffer improves genotyping of single base mutations
- Delivers the benefits of antibody inactivated hot start PCR with SYBR Green detection in a ReadyMix specifically designed for capillary instruments; only primers and template are required
- Protocol is easily scalable, allowing processing of as few as eight to several hundred samples. Assay can be completed in 2.5 hours (30-45 min hands-on time)

Components: Anti-Fluorescein-Peroxidase Conjugate
500× Stock, 300 µl
Denaturation Solution, 100 mL
DNA Dilution Buffer, 250 mL
PlateHyb Hybridization Buffer, 200 mL
Stop Solution, 100 mL
Streptavidin-Coated 8 × 12 Strip-Well Plates, 5 each
Thermoplate Covers, 15 each
TMB Liquid Substrate System, 2 × 100 mL
Wash Buffer Dry Packs (phosphate buffered saline with Tween 20, pH 7.4), 10 each

Storage: 2-8 °C
Shipped in wet ice

Cat. No.	Product Description	Quantity
PPD1	PCR Plate Detection Kit Sufficient for 480 detection reactions	1 kit

Protocol for Microplate Detection of Amplicons