

The Essentials of Custom Diets for Laboratory Animals

What every preclinical investigator needs to know about custom lab animal diets.

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- I. *Custom diets—grain-based and purified.*
- II. *Adding test compounds and medications.*
- III. *"Purified" should mean all ingredients, including casein.*
- IV. *Special diets for the study of obesity, diabetes, and related diseases.*

Abstract. There are several varieties of *custom* diets used in laboratory animal research, among them "direct-add," custom-formulated grain-based, and purified diets. One use of custom diets of all types is the efficacious dosing of test compounds and medications.

The fundamental purposes of a purified diet are full knowledge of all ingredients, complete open formulas, and repeatable consistency, resulting in accurate, reliable data. When using purified diets, the researcher must be assured that all ingredients are truly in a pure form and that the individual components and their energy levels are properly reported.

Many diets are formulated for specific research, for example, the special diets developed for the study of obesity, diabetes, and related diseases. A new TestDiet® series of diet-induced-obesity (DIO) diets delivers up to 70% of the calories from fat and addresses the critical issue of soluble and insoluble fiber content.

Introduction. The TestDiet® division of PMI® Nutrition International/Purina Mills, LLC formulates and produces custom grain-based and purified diets for animal research in academic, government, and pharmaceutical laboratories around the world. We have assembled a team of leading animal nutrition experts who consult with researchers to custom-formulate diets for their specific areas of study. Our nutritionists also conduct extensive independent research in our own laboratories.

We operate the cleanest, most efficient, most comprehensive GMP- and GLP-compatible, ISO-certified lab animal diet production facility in the world to produce any and every custom diet formula presently or previously available from any manufacturer. This guide is designed to provide you, the preclinical researcher, with essential information regarding both grain-based and purified ingredient custom diets.

I. Custom diets—grain-based and purified.

1. Direct Addition to Standard Grain-Based Feed.

We can *add* nutritional components to *standard* grain-based food (such as LabDiet® products). This process is often used for adding fats, oils, cholesterol, and can be used to add any nutrient(s), such as protein, minerals, vitamins, carbohydrate, and fiber.

An important caution—to assure nutritionally sound diets, care must be taken to avoid excessive "dilution" of essential nutrients by adding an overly disproportionate amount of one diet component, thus reducing the relative amount of all the others. For example, if we add 20% fat (by weight) to a standard LabDiet® grain-based product that is 18% protein, the resulting diet will be only 15% protein—the carbohydrates, minerals, vitamins, and fiber have been similarly "diluted." If this dilution factor becomes nutritionally unacceptable, we will recommend producing a nutritionally sufficient custom grain-based formula "from scratch."

2. Custom-Formulated Grain-Based Diet.

We can formulate and produce *custom* grain-based diets "from scratch," using standard multi-nutrient ingredients normally used in such products, or substituting nutrient-specific ingredients (such as milk casein as a protein source).

3. Purified Diets.

"Purified diets," "purified ingredient diets," "semi-purified diets," and "defined diets" (terms which have all come to mean virtually the same thing) are diets produced from nutrient-specific human-grade food sources in which each nutrient is derived from a specific ingredient (*for example*, protein from casein or soy, carbohydrate from corn starch or sucrose, fat from lard or corn oil). Because these purified diets are composed of discrete components, with known and verifiable consistency, from reliable sources, they provide a

precise, repeatable, constantly uniform background to assure trustworthy data. Purified diets provide a dependable base for your research, with unmitigated consistency from batch-to-batch, whether a month, year, or decade later. They are easy to repeat, to modify, and to report.

[N]utritionally defined semipurified diets ... offer several advantages ... including reproducibility and uniformity of content, and the ability to precisely alter composition.... The use of standardized formulations will allow investigators to compare data derived from different laboratories without the concern that unquantifiable differences in the ... diets used contributed to the reported results [Lichtman *et al.*, Hyperlipidemia and Atherosclerotic Lesion Development in LDL Receptor Deficient Mice Fed Defined Semipurified Diets. *Arterioscler Thromb Vasc Biol.* 1999;19:1938, 1942-3].

While our exclusive “Constant Nutrition”™ formulation program assures you of consistent levels of nutrients in our *grain-based* LabDiet® products by varying these natural ingredients as necessary with each batch of diet, nutritional consistency in *purified* diets is obtained differently. Our precise TestDiet® *purified* diets are always produced according to a unique open formula that does not vary from batch to batch. You always know not only the exact nutritional content, but also the specific ingredients and the amounts of each—no more and no less.

There are two schools of thought regarding the composition of purified diet formulas, each well-respected and utilized by researchers worldwide as they deem appropriate for their specific studies: “quantity-balanced” and “calorie-balanced.” (Calorie-balanced diets are particularly useful obesity and diabetes research. These formula differences are discussed in section IV, below.)

II. Adding test compounds and medications.

Adding test compounds to standard and custom grain-based diets and to purified diets is an effective, accurate, and convenient means of dosing your animals for research purposes. With precision and consistency, we can homogeneously blend in virtually any compound, medication, or nutrient and produce the diet in any form: pellets, meal, or tablets; many can be made into a powder form for liquid diets. We do **not** add toxins, carcinogens, mutagens, radioactive substances, controlled dangerous substances (CDS), or live organisms (viruses, bacteria, etc.).

III. “Purified” should mean *all* ingredients, including casein.

Whoever manufactures your purified diets should assure you that they are using pure ingredients of known, verifiable, consistent composition to the greatest extent practicable.

For example, a number of purified diet manufacturers use in their diets less-expensive milk casein that is *not* vitamin-free; consequently, unknown and random amounts of various vitamins are introduced into each batch of diet without being recognized or reported.

The protein content of this less expensive casein can also fluctuate significantly. This cost-cutting practice defeats the fundamental purposes of a purified diet—full knowledge of all ingredients, complete open formulas, and repeatable consistency, resulting in accurate, reliable data.

Consistent with the value of your research, we at the PMI® TestDiet® division use **only** the more expensive, more precise vitamin-free milk casein in our purified diets; and, we routinely assay our casein to confirm that the protein content is within tight tolerances.

When consistent diets go into your research, precise results come out.™ Purified diets are a major investment in the quality and reliability of your research. Typically, post-manufacturing analysis is not conducted on purified diets, but product integrity depends upon the quality and consistency of the ingredients and the integrity of the manufacturing process. We operate the cleanest, most efficient, most precise purified diet production laboratory in the world. Our facilities are GLP & GMP compatible, and ISO-certified. Because of these certifications, our depth of expertise in lab animal nutrition, our scrupulous attention to detail and precision, and our commitment to using the finest ingredients regardless of cost, you never need to conduct post-production analysis for a TestDiet® product. You should hold whoever produces your custom diets to these same exacting standards.

IV. Special diets for the study of obesity, diabetes, and related diseases.

PMI® TestDiet® division produces a multitude of formulas for obesity and diabetes research—and we will produce custom variations to meet your specific research needs.

One series of lab animal research diets that has been used widely is the “van Heek” series—known as “D12450B,” “D12451,” and “D12492.” Other manufacturers of these diets present them as providing

10%, 45%, and 60% kcal from fat, respectively. In fact, this presentation is *erroneous*, because casein is reported as being 100% protein, when it is actually only about 90% protein. (See *chart on page 4*; see also TestDiet® *descriptions on page 8*.)

These three formulas—"D12450B," "D12451," and "D12492"—are available from TestDiet® exactly as originally formulated but at more reasonable cost. We have also continued this series with a **70% kcal fat** diet with the same micronutrients, fiber, and protein. *For the corresponding TestDiet® product numbers, see the charts on pages 4 and 5; see also the TestDiet® descriptions on page 8.*

TestDiet® DIO Series™

TestDiet® also produces the "**TestDiet® DIO Series™**" of diets for obesity, diabetes, and related studies based upon our globally-respected Basal Diet #5755. In the **TestDiet® DIO Series™**, all the micronutrients, proteins, and fiber are **balanced by calories**—the only variation being relative caloric contribution of fats and carbohydrates, with 12%*, 45%, 60%, and 70% kcal from fat (or whatever other variation you would like). When an animal has eaten the same amount of calories of any diet, it will have ingested the same amount of protein, fiber, and micronutrients.

This series also recognizes the growing attention to the **function of fiber** in metabolism of fats by providing equal parts of the **insoluble fiber** traditionally used in purified diets (cellulose) and **soluble fiber** (inulin), to more closely resemble a natural ingredient diet. (See *chart on page 6*; see TestDiet® *descriptions on page 7*.) We can also adjust the amount and source of fats and carbohydrates to achieve any variation of these diets as necessary for your specific needs. All of these diets are typically produced as pellets, but they are available in powder/meal or "cookie dough" form as well.

"Calorie-Balanced" and "Quantity-Balanced"

As mentioned above, purified diets are usually formulated from either a "quantity-balanced" or a "calorie-balanced" perspective, each well-respected and utilized by researchers worldwide. Calorie-balanced formulas are most commonly used for obesity and diabetes research. Following are explanations of both of these concepts.

Calorie-Balanced.

Many purified diets are designed to provide consistent nutrition for the same amount of **calories** consumed, regardless of ingredient modifications.

These diets deliver the same micronutrients and fiber for each calorie consumed, even though as one or more of the energy sources may vary, quantity of diet may increase or decrease. (As one or another source of energy is adjusted, the amount of the fiber component is *not* adjusted, but remains constant.) Typically, for each calorie consumed, the ingested amount of minerals, vitamins, fiber, and one of the three energy sources will remain constant.

For example, in a diet-induced obesity series of diets, an animal may consume 2.1 gm of casein (90% protein), 0.2 gm of vitamin mix, and 0.5 gm of calcium for each 41 calories, although it may require more than 10 gm of a low-fat diet and only about 7 gm of a very-high-fat diet to deliver the same amount of calories. (See *examples on pages 4-6, below*.)

Quantity-Balanced.

Quantity-balanced formulas are designed to provide consistent nutrition in like **quantities** of food no matter how the ingredients may be varied. In these diet formulas, the diet will deliver the same amount of each micronutrient—vitamins and minerals—and the same amount of energy—calories (kcal)—for each gram of food. Typically, as one or another source of energy (fat, carbohydrate, or protein) is adjusted, the fiber content is also adjusted.

For example, a series of diets may each deliver 4080 kcal per kg, 1.4 gm of choline per kg, and 6 gm of calcium per kg, even though the calories derived from fat may increase, the calories from carbohydrates decrease, and the fiber content increase. (Fiber would increase because fat is more calorie-dense than protein and carbohydrate; consequently, more fiber must be added to maintain the quantity or weight of the diet as carbohydrate calories are replaced by fat calories.) (See TestDiet® *Basal Formula 5755 on page 7 as an example of a "quantity-balanced" diet*.)

PMI® TestDiet® division produces both "Quantity-Balanced" and "Calorie-Balanced" purified diet formulas based upon your particular research needs.

*We believe that a "control" diet with 12% kcal from fat is more consistent with the generally-accepted standards of nutrition for rodents and is more compatible with usual and customary lab rodent grain-based feed (e.g., LabDiet® 5001) than is a control deriving only 10% kcal from fat.

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Original "van Heek" formulas showing erroneous kcal calculations of other manufacturers and accurate calculations as corrected by PMI® TestDiet® division—

½" Pellet Available as meal also	"10% kcal fat" (nominal)				"45% kcal fat" (nominal)				"60% kcal fat" (nominal)			
	<i>Original</i> D12450B		TestDiet® #58Y2		<i>Original</i> D12451		TestDiet® #58V8		<i>Original</i> D12492		TestDiet® #58Y1	
	<i>yellow</i> Accurate kcal calculations, corrected by PMI TestDiet®		<i>pink</i> Accurate kcal calculations, corrected by PMI TestDiet®		<i>blue</i> Accurate kcal calculations, corrected by PMI TestDiet®		Erroneous kcal calculation by other manufacturers		Erroneous kcal calculation by other manufacturers		Erroneous kcal calculation by other manufacturers	
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	49.2	20.0	17.3	18.3	23.7	20	21.3	18.3	26.2	20	23.6	18.3
Carbohydrate	67.3	70.0	67.7	71.6	44.4	35	41.9	36.0	26.3	20	26.8	20.8
Fat	4.3	10.0	4.3	10.2	23.6	45	23.6	45.7	34.9	60	34.9	60.9
kcal/gm	3.86				4.75				5.26			
		3.78				4.65				5.16		
Ingredient	gm per 1.055 kg	kcal	kcal	gm/kg	gm per .858 kg	kcal	kcal	gm/kg	gm per .774 kg	kcal	kcal	gm/kg
Casein	200	800 721	683.42	189.56	200	800 721	840.22	233.06	200	800 721	931.76	258.45
L-Cystine	3	12	11.37	2.84	3	12	13.98	3.50	3	12	15.51	3.88
Corn Starch	315	1260	1194.26	298.56	72.8	291	339.34	84.83	0	0	0.00	0.00
Maltodextrin 10	35	140	132.70	33.17	100	400	466.12	116.53	125	500	646.12	161.53
Sucrose	350	1400	1326.95	331.74	172.8	691	805.45	201.36	68.8	272	355.62	88.91
Cellulose	50	0	0.00	47.39	50	0	0.00	58.27	50	0	0.00	64.61
Soybean Oil	25	225	213.26	23.70	25	225	262.19	29.13	25	225	290.75	32.31
Lard	20	180	170.60	18.96	177.5	1598	1861.56	206.84	245	2205	2849.39	316.60
Mineral Mix (S10026)	10	16	15.16	9.48	10	16	18.64	11.65	10	16	20.68	12.92
DiCalcium Phosphate	13	0	0.00	12.32	13	0	0.00	15.15	13	0	0.00	16.80
Calcium Carbonate	5.5	0	0.00	5.21	5.5	0	0.00	6.41	5.5	0	0.00	7.11
Potassium Citrate, 1 H ₂ O	16.5	0	0.00	15.64	16.5	0	0.00	19.23	16.5	0	0.00	21.32
Vitamin Mix (V10001)	10	40	37.09	9.48	10	40	45.61	11.65	10	40	50.57	12.92
Choline Bitartrate	2	0	0.00	1.90	2	0	0.00	2.33	2	0	0.00	2.58
FDC Yellow #5	0.05	0	0.00	0.05	0	0	0.00	0.00	0	0	0.00	0.00
FDC Red #40	0	0	0.00	0.00	0.05	0	0.00	0.06	0	0	0.00	0.00
FDC Blue #1	0	0	0.00	0.00	0	0	0.00	0.00	0.05	0	0.00	0.07
Total	1055.05 grams deliver	4073 3994 kcal	3784.82	1000	858.15 grams deliver	4073 3994 kcal	4653.12	1000	773.85 grams deliver	4070 3991 kcal	5160.40	1000
			per kg				per kg				per kg	

See page 8 for chemical composition and typical analysis of the TestDiet® versions of these diets.

Continuation of "van Heek" series with 70% kcal (actual) from fat—

Continuation of "van Heek" series				
70% kcal fat (actual)				
	This formula not offered by other manufacturers of "van Heek" series of diets		TestDiet® #58G6 <i>green</i> Available only as meal, "cookie dough" consistency.	
Protein			<i>gm%</i> 25.2	<i>kcal%</i> 18.3
Carbohydrate			16.3	11.8
Fat			42.8	69.9
kcal/gm			5.52	
Ingredient	gm	kcal	kcal	gm/kg
Casein	200	721.04	995.84	276.22
L-Cystine	3	12.00	16.57	4.14
Corn Starch	0	0.00	0.00	0.00
Maltodextrin 10	90	360.00	497.20	124.30
Sucrose	14	56.00	77.34	19.34
Cellulose	50	0	0.00	69.06
Soybean Oil	25	225.00	310.75	34.53
Lard	285	2565.00	3542.57	393.62
Mineral Mix (S10026)	10	16.00	22.10	13.81
DiCalcium Phosphate	13	0	0.00	17.96
Calcium Carbonate	5.5	0	0.00	7.60
Potassium Citrate, 1 H ₂ O	16.5	0	0.00	22.79
Vitamin Mix (V10001)	10	39.14	54.05	13.81
Choline Bitartrate	2	0	0.00	2.76
FD&C Yellow #5	0	0	0.00	0.00
FD&C Red #40	0	0	0.00	0.00
FD&C Blue #1	0.05	0	0.00	0.07
Total	724.05 grams deliver	3994 kcal	5516.44 <i>per kg</i>	1000

See page 8 for chemical composition and typical analysis of the TestDiet® version of this diet.

“TestDiet® DIO Series”™

	TestDiet® #58G7 ½” Pellet Available also as meal (powder) 12% kcal fat light yellow/white			TestDiet® #58G8 ½” Pellet Available also as meal (powder) 45% kcal fat pink			TestDiet® #58G9 ½” Pellet Available also as meal (powder) 60% kcal fat blue			TestDiet® #58H0 Meal ("cookie dough" consistency) 70% kcal fat green		
	gm%	kcal	kcal%	gm%	kcal	kcal%	gm%	kcal	kcal%	gm%	kcal	kcal%
Protein	20.01%	763.0	18.7	24.31%	763.0	18.7	26.95%	763.0	18.7	29.06%	763.0	18.7
Carbohydrate	66.98%	2831.5	69.3	42.68%	1485.0	36.4	27.79%	872.2	21.4	15.86%	461.8	11.3
Fat	5.16%	490.5	12.0	23.46%	1837.0	45.0	34.69%	2449.8	60.0	43.67%	2860.2	70.0
Minerals & Vitamins	3.79%			4.61%			5.11%			5.51%		
Fiber	4.06%			4.93%			5.47%			5.89%		
Total kcal/gm	100%	4085.0	100.0	100%	4085.0	100.0	100%	4085.0	100.0	100%	4085.0	100.0
		3.87			4.70			5.21			5.61	
Ingredient	gm	kcal	gm/kg	gm	kcal	gm/kg	gm	kcal	gm/kg	gm	kcal	gm/kg
Casein, Vitamin-Free	210.0	757.0	198.6990	210.0	757.0	241.4173	210.0	757.0	267.6011	210.0	757.0	288.5606
DL-Methionine	1.5	6.0	1.4193	1.5	6.0	1.7244	1.5	6.0	1.9114	1.5	6.0	2.0611
Dextrin	513.9	2055.5	486.2212	262.3	1049.0	301.4877	149.1	596.2	189.9331	71.5	285.8	98.1793
Sucrose	175.0	700.0	165.5825	90.0	360.0	103.4646	50.0	200.0	63.7146	25.0	100.0	34.3525
Cellulose (insoluble fiber)	15.0	0.0	14.1928	15.0	0.0	17.2441	15.0	0.0	19.1144	15.0	0.0	20.6115
Inulin (soluble fiber)	15.0	0.0	14.1928	15.0	0.0	17.2441	15.0	0.0	19.1144	15.0	0.0	20.6115
Corn Oil	50.0	450.0	47.3093	50.0	450.0	57.4803	50.0	450.0	63.7146	50.0	450.0	68.7049
Lard	4.5	40.5	4.2578	154.1	1387.0	177.1658	222.2	1999.8	283.1475	267.8	2410.2	367.9835
RP Mineral Mix #10 (25.8% fiber)	50.0	0.0	47.3093	50.0	0.0	57.4803	50.0	0.0	63.7146	50.0	0.0	68.7049
Choline Chloride	2.0	0.0	1.8924	2.0	0.0	2.2992	2.0	0.0	2.5486	2.0	0.0	2.7482
RP Vitamin Mix (95% sucrose)	20.0	76.0	18.9237	20.0	76.0	22.9921	20.0	76.0	25.4858	20.0	76.0	27.4820
FD&C color	trace		trace	trace		trace	trace		trace	trace		trace
Total	1057 grams deliver		1000	870 grams deliver		1000	785 grams deliver		1000	728 grams deliver		1000
	4085 kcal			4085 kcal			4085 kcal			4085 kcal		

The “TestDiet® DIO Series”™ of diets for obesity, diabetes, and related studies based upon our globally-respected Basal Diet #5755. In the TestDiet® DIO Series™, all the micronutrients, proteins, and fiber are **balanced by calories**—the only variation being relative caloric contribution of fats and carbohydrates, with 12%*, 45%, 60% and 70% kcal from fat (or whatever other variation you would like). When an animal has eaten the same amount of calories of any diet, it will have ingested the same amount of protein, fiber, and micronutrients. This series also recognizes the growing attention to the function of fiber in metabolism of fats by providing equal parts of the **insoluble fiber** traditionally used in purified diets (cellulose) and **soluble fiber** (inulin), the carbohydrates more closely resembling a varied natural ingredient diet. We can also adjust the amount and source of fats and carbohydrates to achieve any variation of these diets as necessary for your specific needs. All these diets are typically produced as extruded pellets (including the 70% kcal fat), but they are available in powder/meal form as well. *We believe that a “control” diet with 12% kcal from fat is more consistent with the generally-accepted standards of nutrition for rodents and is more compatible with usual and customary lab rodent grain-based feed (e.g., LabDiet® 5001) than is a 10% kcal fat control diet.

	TestDiet® DIO Series™				Standard Basal Diet #5755 ½" Pellet or Meal
	12% kcal fat DIO Basal Diet #58G7 ½" Pellet or Meal	45% kcal fat #58G8 ½" Pellet or Meal	60% kcal fat #58G9 ½" Pellet or Meal	70% kcal fat #58H0 Meal ("cookie dough" consistency)	
INGREDIENTS	light yellow/white	pink	blue	green	
Casein-Vitamin Free	19.8700	24.1420	26.7600	28.8560	21.00
DL-Methionine	0.1419	0.1724	0.1911	0.2061	0.15
Sucrose	16.5580	10.3465	6.3715	3.4353	15.00
Dextrin	48.6222	30.1482	18.9932	9.8182	43.65
Corn Oil	4.7310	5.7480	6.3715	6.8705	3.00
Lard	0.4257	17.7170	28.3150	36.7980	5.00
RP Mineral Mix #10	4.7310	5.7480	6.3715	6.8705	5.00
Choline Chloride	0.1892	0.2299	0.2548	0.2748	0.20
RP Vitamin Mix	1.8924	2.9920	2.5486	2.7482	2.00
Powdered Cellulose	1.4193	1.7244	1.9114	2.0612	3.00
Inulin	1.4193	1.7244	1.9114	2.0612	0.00
CHEMICAL COMPOSITION¹					
Nutrients²					
Protein, %	18.00	21.90	24.2	26.1	19.5
Fat, %	5.20	23.50	34.70	43.70	10
Cholesterol, ppm	4.00	168.00	269.00	350.00	
Fiber (max), %	4.10	4.90	5.50	5.90	4.3
Carbohydrates, %	67.00	42.70	27.80	15.90	60.6
Energy², kcal/gm	3.87	4.70	5.21	5.62	4.08
Minerals					
Calcium, %	0.57	0.69	0.77	0.83	0.600
Phosphorous, %	0.54	0.65	0.72	0.78	0.570
Potassium, %	0.38	0.46	0.51	0.55	0.400
Magnesium, %	0.07	0.08	0.09	0.09	0.069
Sodium, %	0.20	0.24	0.27	0.29	0.210
Chlorine, %	0.23	0.27	0.30	0.33	0.240
Flourine, ppm	4.70	5.70	6.30	6.80	5.000
Iron, ppm	57.00	69.00	77.00	83.00	60.000
Zinc, ppm	20.00	24.00	26.00	28.00	20.000
Manganese, ppm	62.00	75.00	83.00	89.00	65.000
Copper, ppm	14.20	17.30	19.10	20.60	15.000
Cobalt, ppm	3.00	3.70	4.10	4.40	3.200
Iodine, ppm	0.54	0.66	0.73	0.79	0.600
Chromium, ppm	2.90	3.50	3.90	4.20	3.000
Molybdenum, ppm	0.77	0.94	1.04	1.12	0.800
Selenium, ppm	0.22	0.26	0.29	0.31	0.200
Vitamins					
Vitamin K (menadione), ppm	9.84	11.96	13.25	14.29	10.4
Thiamine Hydrochloride, ppm	19.50	23.70	26.30	28.30	20.0
Riboflavin, ppm	18.90	23.00	25.50	27.50	20.0
Niacin, ppm	85.00	103.00	115.00	124.00	90.0
Pantothenic Acid, ppm	52.00	63.00	70.00	76.00	55.0
Choline Chloride, ppm	1,324.00	1,609.00	1,784.00	1,924.00	1,400.00
Folic Acid, ppm	3.80	4.60	5.10	5.50	4.0
Pyrodoxine, ppm	15.60	18.90	21.00	22.60	16.0
Biotin, ppm	0.40	0.50	0.50	0.50	0.4
Vitamin B-12, mcg/kg	19.00	23.00	25.00	27.00	20.0
Vitamin A, IU/g	20.90	25.40	28.20	30.40	22.0
Vitamin D-3 (added), IU/g	2.10	2.50	2.80	3.00	2.2
Vitamin E, IU/kg	47.40	57.50	63.80	68.80	50.0
Ascorbic Acid, ppm	0.00	0.00	0.00	0.00	0.0
Calories provided by:					
Protein, %	18.623	18.623	18.623	18.623	19.00
Fat (ether extract), %	12.009	44.978	59.982	70.029	10.00
Carbohydrates, %	69.3267	36.399	21.395	11.348	60.60

Diet-Induced-Obesity Rodent Purified Diets based on TestDiet® Basal Diet Formula

Storage conditions are particularly critical to TestDiet® products, due to the absence of antioxidants or preservative agents. To provide maximum protection against possible changes during storage, store in a dry, cool location. Storage under refrigeration (2 degrees C) is required. If long term studies are involved, store the diet at -20 degrees C or colder. Be certain to keep in air tight containers.

Feeding Directions: Feed ad libitum. Plenty of fresh, clean water should be available at all times.

¹Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly.

²Physiological Fuel Value (kcal/gm) - Sum of decimal fractions of protein, fat, and carbohydrate (use Nitrogen Free Extract) x 4, 9, 4 kcal/gm, respectively.

"van Heek" DIO Series	"10% kcal fat" "nominal" TestDiet® #58Y2 ½" Pellet or Meal "D12450B"	"45% kcal fat" "nominal" TestDiet® #58V8 ½" Pellet or Meal "D12451"	"60% kcal fat" "nominal" TestDiet® #58Y1 ½" Pellet or Meal "D12492"	70% kcal fat actual TestDiet® #58G6 Meal ("cookie dough") N/A
<i>Compare to</i>	<i>yellow</i>	<i>pink</i>	<i>blue</i>	<i>green</i>
INGREDIENTS				
Casein-Vitamin Free	18.9560	23.3060	25.8450	27.6220
L-Cystine	0.2840	0.3500	0.3880	0.4140
Dextrin (Corn Starch)	29.8560	8.4830	0.0000	0.0000
Maltodextrin	3.3170	11.6530	16.1530	12.4300
Sucrose	33.1740	20.1360	8.8910	1.9330
Cellulose	4.7390	5.8270	6.4610	6.9060
Soybean Oil	2.3700	2.9130	3.2310	3.4530
Lard	1.8960	20.6840	31.6600	39.3620
DIO Mineral Mix	0.9480	1.1650	1.2920	1.3810
DiCalcium Phosphate	1.2320	1.5150	1.6800	1.7960
Calcium Carbonate	0.5210	0.6410	0.7110	0.7600
Citric Acid	1.5640	1.9230	2.1320	2.2790
AIN-76A Vitamin Mix	0.9480	1.1650	1.2920	1.3810
Choline Bitartrate	0.1900	0.2330	0.2580	0.2760
FD&C Color	0.0050	0.0060	0.0060	0.0070
CHEMICAL COMPOSITION¹				
Nutrients²				
Protein, %	17.30	21.30	23.60	25.2
Fat, %	4.30	23.60	34.90	42.80
Cholesterol, ppm	18.00	196.00	301.00	374.00
Fiber (max), %	4.70	5.80	6.50	6.90
Carbohydrates, %	67.70	41.90	26.80	16.30
Energy², kcal/gm	3.78	4.65	5.16	5.52
Minerals				
Calcium, %	0.57	0.70	0.78	0.83
Phosphorous, %	0.43	0.53	0.59	0.63
Potassium, %	0.57	0.70	0.77	0.82
Magnesium, %	0.05	0.06	0.07	0.07
Sodium, %	0.10	0.12	0.13	0.14
Chlorine, %	0.15	0.18	0.20	0.22
Flourine, ppm	0.90	1.10	1.20	1.20
Iron, ppm	44.00	54.00	60.00	64.00
Zinc, ppm	28.00	34.00	38.00	40.00
Manganese, ppm	55.00	68.00	76.00	81.00
Copper, ppm	5.70	7.00	7.80	8.30
Cobalt, ppm	0.00	0.00	0.00	0.00
Iodine, ppm	0.20	0.24	0.27	0.29
Chromium, ppm	1.90	2.30	2.60	2.80
Molybdenum, ppm	1.55	1.90	2.11	2.25
Selenium, ppm	0.15	0.19	0.21	0.22
Vitamins				
Vitamin K (menadione), ppm	0.48	0.58	0.65	0.69
Thiamine Hydrochloride, ppm	5.70	7.00	7.80	8.30
Riboflavin, ppm	5.70	7.00	7.80	8.30
Niacin, ppm	28.00	35.00	39.00	41.00
Pantothenic Acid, ppm	14.00	17.00	19.00	20.00
Choline Chloride, ppm	950.00	1,165.00	1,290.00	1,380.00
Folic Acid, ppm	1.90	2.30	2.60	2.80
Pyrodoxine, ppm	5.50	6.70	7.40	8.00
Biotin, ppm	0.20	0.20	0.30	0.30
Vitamin B-12, mcg/kg	9.00	12.00	13.00	14.00
Vitamin A, IU/g	3.80	4.70	5.20	5.50
Vitamin D-3 (added), IU/g	0.90	1.20	1.30	1.40
Vitamin E, IU/kg	47.40	58.30	64.60	69.10
Ascorbic Acid, ppm	0.00	0.00	0.00	0.00
Calories provided by:				
Protein, %	18.290	18.290	18.291	18.286
Fat (ether extract), %	10.153	45.680	60.903	69.912
Carbohydrates, %	71.557	36.027	20.807	11.802

**Diet-Induced-Obesity
Rodent Purified Diets
based on
AIN-76A
Diet Formula**

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